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THE RECIPROCAL EFFECTS OF NITROGEN, PHOSPHORUS, AND POTASSIUM AS RELATED TO THE ABSORPTION OF THESE ELEMENTS BY PLANTS[1]

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The Pennsylvania State College

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INTRODUCTION

Importance of understanding energy relations between soil and plant

Coincident with the rapid advances in physical chemistry, concepts with respect to soil fertility problems are undergoing profound changes. This is manifested by the results of recent investigations (1, 5, 8, 9, 25, 28, 83, 95) in which the principles of plant production are shown to be dependent not only upon a knowledge of the exchanges of energy in the soil, but also upon the energy relations of chemical reactions between the soil and the plant, including necessarily the transformations of energy taking place within the living cells. This paper will treat of the factors affecting the equilibria of nitrogen, phosphorus, and potassium in the soil in relation to the absorption of these elements by the plant.

A mathematical expression for evaluating the factors of fertility

It will simplify our presentation of the subject of the so-called "selective" absorption of nutrient elements from the soil if we can find an expression that will enable us to visualize the dominant soil factors affecting the nutrition of plants (35).

Influence of water.—Water is without question the greatest limiting factor in plant development. It is known from numerous experiments, especially from those of Hellriegel (19) and of Wollny (94), that for maximum growth there exists for each soil type an optimum water content. If Hellriegel's experimental results (19) on the variation of dry matter produced as a function of the water content of the soil are plotted, it is found that the points lie approximately on a parabola. Let A (fig. 1) represent the yield when the water content of the soil is optimum and let Y represent the actual yield. Since the curve obtained is a parabola,

$$\therefore k(x_1 - x_0)^2 = A - Y \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (A)$$

[1] Presented before the soil fertility conference held in commemoration of the 50th Anniversary of the Old Fertility Plots of the Pennsylvania State College. Publication authorized by the director of the Pennsylvania Agricultural Experiment Station as technical paper no. 534.

i.e., the decrease in yield from the maximum is proportional to the deviation in the water content of the soil from the optimum, or

$$Y = A - k(x_1 - x_0)^2 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (B)$$

where k is a constant expressing the sensitiveness of the plant to changes in the water content of the soil.

Water content of the soil in relation to the maximum efficiency of mineral nutrients.—But Woilny's experiments (93) show that the optimum water content of the soil corresponds also to the maximum efficiency of the mineral nutrients, i.e., to their maximum absorption. Since growth from balanced nutrient solutions is a function of the amount of the nutrient element absorbed, therefore, the absorption of a nutrient element is also proportional to

$$A - k(x_1 - x_0)^2 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (C)$$

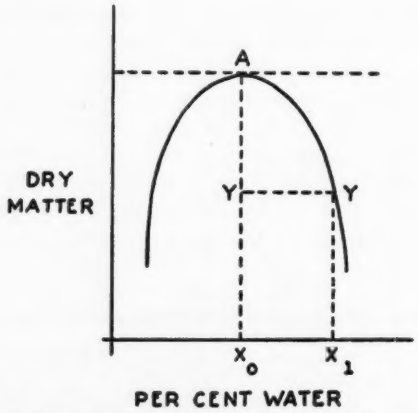


FIG. 1. RELATION OF YIELD TO WATER CONTENT OF SOIL

Let S_t represent the quantity of soil explored by the roots within a period of time t. Also let $\frac{1}{s}$ represent that fraction of the soil mass considered (S_t) entering into solution per unit of time in the form of a specific nutrient element (nitrogen, phosphorus, or potassium); and let $\frac{1}{r}$ be the fraction absorbed per unit of time by the plant. Then in a unit of time the fraction of the soil mass absorbed by the plant will be $\frac{S_t}{sr}$, where s measures the resistance to solution and r measures the physiological resistance of the roots to the absorption of the dissolved element (osmosis). The former can vary from $+\infty$ to 1. It is always great and depends on the nature of the soil. The latter can vary from $+\infty$ to 1 and depends on the feeding power of the plant (84, 89, 90, 91).

From (*C*) the absorption per unit of time is proportional to

$$A - k(x_1 - x_0)^2 \quad \ldots\ldots (D)$$

Let us put

$$\frac{1}{sr} = K\left\{A - k(x_1 - x_0)^2\right\} \cdot \frac{1}{s_1 r_1} \quad \ldots\ldots (E)$$

The expression on the right represents then the fraction of the soil that passes from the solid condition to the plant in a unit of time.

Then, as we increase the time from t to $t + dt$, the amount absorbed (dy) will be

$$dy = K\left\{A - k(x_1 - x_0)^2\right\} \cdot \frac{S_t}{s_1 r_1} \cdot dt \quad \ldots\ldots (F)$$

where K is a constant representing the capacity of the plant. Or for the rate of absorption

$$\frac{dy}{dt} = K\left\{A - k(x_1 - x_0)^2\right\} \cdot \frac{S_t}{s_1 r_1} \quad \ldots\ldots (G)$$

In experimental work, of course, (dt) is relatively large; but it is remarkable that the average rate of absorption remains relatively constant for long periods of time (15 days, for example).

The equation shows that a soil can be deficient with respect to a given nutrient element for two reasons: (a) Inability, during periods of very active absorption, to maintain the supply above critical concentrations of the element. The function of fertilizers is to create a potential supply to provide for these emergencies. (b) Inability to provide at any time the supply of an essential element above critical concentrations. In this category are worn out or run down soils.

The equation modified by the addition of fertilizers.—Similarly for the rate of absorption of an element introduced in a soluble form such as a fertilizer which modifies a mass (say S') of the soil, it may be shown that the rate of absorption of the element introduced is expressed by the equation

$$\frac{dy}{dt} = K'\left\{A' - k'(x_0 - x_1)^2\right\} \cdot \frac{S'_t}{s_1' r_1'} \quad \ldots\ldots (H)$$

The complete equation expressing the influence of the soil and of the added fertilizer is, therefore,

$$\frac{dy}{dt} = \underbrace{K\left\{A - k(x_0 - x_1)^2\right\} \cdot \frac{S_t}{s_1 r_1}}_{\text{(fertility factor)}} + \underbrace{K'\left\{A' - k'(x_0 - x_1)^2\right\} \cdot \frac{S'_t}{s_1' r_1'}}_{\text{(fertilization factor)}} \quad \ldots\ldots (I)$$

The formula clearly indicates the dominent rôle exercised by a soluble fertilizer in plant nutrition. As we shall see later the reciprocal influence of one element (ion) on another will involve the terms s and r of this equation.

The earliest experiments on the reciprocal effect of elements on absorption by plants

The reciprocal or mutual effect of one element upon another was first observed about the middle of the last century by Wolff (92). In his extensive nutrient solution culture investigations on the growth of barley, Wolff observed that the yield of plants grown in his "complete" nutrient solution was greater than that from cultures in which sodium was omitted. But Wolff's nutrient solutions were too high in potassium to be well balanced with respect to the concentration of this element. Analyses of the plants showed that the introduction of the sodium ion—from sodium chloride—decreased the absorption of potassium and so prevented its luxury consumption ("luxuskonsumption"), an expression which we have heard much about recently. Thus was it learned that absorption of an element in excess of that utilized might reduce yields and that this absorption in excess of the amount of the element required for the production of a normal plant could be controlled by the addition of another element—in Wolff's experiments, sodium. These experiments of Wolff's with sodium and potassium were confirmed by a number of investigators and definitely established the reciprocal action of at least two elements.

The concept of physiological balance

The existence of antagonism between other elements.—This reciprocal action of one element resulting in suppressing the absorption of another was later found to occur between other elements (ions) in the case of absorption by both plant and animal cells. A reciprocal relationship was found to exist between Ca^{++} and K^{+} (10, 22, 27, 56), between NH_4^{+} and K^{+} (48, 49, 50), between Sr^{++} and K^{+} (55), between Ca^{++} and Mg^{++} (51), between Na^{+} and NH_4^{+} (65, 66, 67), between SiO_2^{--} and PO_4^{---} (5, 16, 18, 43, 44), between Mg^{++} and Sr^{++} (55), between Mg^{++} and Ba^{++} (55), between Na^{+} and Ca^{++}, between Na^{+} and Mg^{++} (22, 27) and between K^{+} and Fe^{+++} (15, 29).

Diminution of antagonistic action with dilution.—Loeb (49) gave the term "antagonism" to this phenomenon. But Osterhout (68) showed later that the antagonistic action increased with the increase in the concentration of the solution. In very dilute solutions, such as that of the order of magnitude obtained from soils by the various displacement methods, no antagonistic effect was observed. It follows that if the same mechanism is operative in soils as in nutrient culture solutions, it must be postulated that plant roots obtain their nutrients from a much more concentrated solution than that obtained thus far by artificial means.

Optimum ratios.—These investigations by Loeb and Osterhout led to the development of the concept of physiologically balanced solutions, from which followed the long search by means of nutrient culture solutions to discover the

best (optimum) ratios between the various nutritive elements (7, 20, 32, 47, 52, 53, 54, 73, 74, 76, 77, 78, 79, 88).

Nutrient culture versus field experiments

Absorption from soil and nutrient solutions compared.—The principles acquired by means of these laboratory studies cannot be overestimated with respect to the service rendered in providing a system of physiological knowledge for application to field studies. The environmental conditions, however, affecting the absorption of elements from the soil are different from those under which nutrient culture experiments are conducted because: (a) The rate of diffusion of ions toward the root zones is more rapid in nutrient culture solutions than in the soil (21); (b) the extent of the root system and, therefore, the absorbing surface is greater under field conditions than in nutrient culture solutions. The factor $\frac{1}{s_i r_i}$ of equation (*I*) thus becomes unity in solution but not in soil cultures.

Soil versus plant analysis.—Because the concentration of the soil solution with respect to any of the principal elements is subject to wide fluctuations even in the uncropped condition (2, 3, 80), it is difficult to draw conclusions relative to absorption of ions from soil solution studies alone. It is possible, however, as we shall see by comparing the amounts of nutrients absorbed—at the different vegetative periods—by plants growing under uniform soil and meteorological conditions, to extend very considerably our knowledge of the factors influencing the absorption of the principal (dominant) elements (ions) from soils.

THE LAW OF THE MINIMUM AND ITS IMPLICATION

Many "laws" have been advanced purposing to define the relationship between the amount of nutrients absorbed by the plant and the dry matter produced (45, 46, 60). The first was by Liebig (45), who announced as a corollary to what afterwards became known as the "law of the minimum" that nutrient elements must be absorbed in certain definite proportions and in such a manner that when the rate of supply of one of the principal nutrient elements is reduced below the critical concentration for that element the rate of absorption of the other principal nutrient elements is retarded or depressed, because an inadequate supply will render the other elements unavailable, since the plant will be unable to grow to any extent. Consequently, assuming all the other growth factors constant, it follows from Liebig's basic hypothesis that if, with time as the abscissa and the quantity of nutrient element absorbed during the growth of a plant as ordinate, graphs are plotted indicating the course of absorption of nitrogen, phosphorus, and potassium from a soil to which a "complete" fertilizer has been added—with all other growth factors constant—the graph showing the course of absorption of any one of these elements from a fertilizer containing only two of them would be displaced from its former position and would now fall below it.

The results of field experiments

The American field experiments.—The results which we have obtained in our experiments on the absorption of these elements by apple trees grown in the field under controlled conditions (86) are shown graphically in figure 2. The data are expressed in percentage of the element absorbed by the last season's branch growth on a moisture-free basis. These results are in accordance with Liebig's basic hypothesis. Thus, it will be observed that the omission of any one of the elements—nitrogen, phosphorus, or potassium—from the fertilizer has resulted in a decreased absorption of the remaining elements and is accompanied by decreased growth and reproduction. Analytical data recently

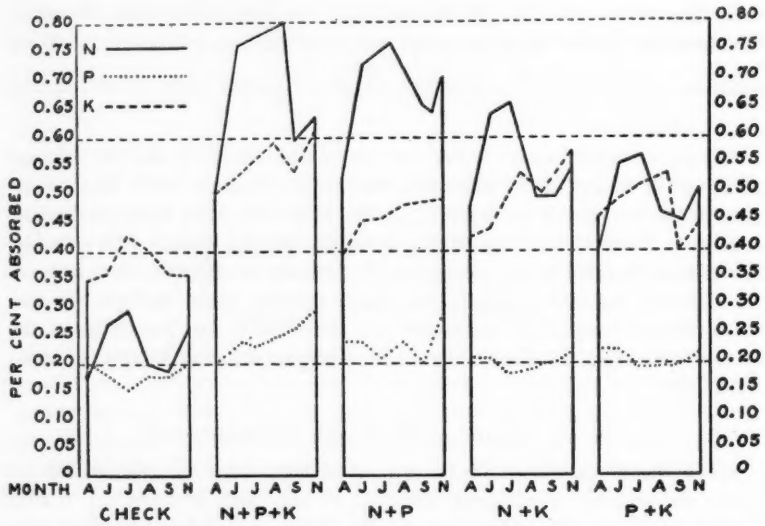


FIG. 2. THE COURSE OF THE ABSORPTION OF NITROGEN, PHOSPHORUS, AND POTASSIUM BY *Pyrus malus L.* AS DETERMINED BY THE PERIODIC ANALYSIS OF THE CURRENT AND SEASON'S BRANCH GROWTH

Results are expressed in percentages of dry weight of material

obtained with respect to the total amount of each element taken up by an entire tree show the same relationships.

The French field experiments.—But the results of very carefully conducted long time experiments by Lagatu and Maume (34, 36, 37, 38, 40) in Montpelier on the course of absorption of nitrogen, phosphorus, and potassium by the vine (*Vitis vinifera*) show that in wet as well as dry years the omission of one of the three elements—nitrogen, phosphorus, potassium—from the fertilizer resulted not in a decreased absorption of the remaining elements relative to that from the complete fertilizer, as would be expected from Liebig's law, but on the contrary in decreased yields produced by a nutritional lack of balance caused

by an *increase* in the absorption of the remaining elements of the fertilizer. The results shown in figure 3 are given in percentages of the dry weights of leaves.

These results are of paramount importance in the science of plant and animal nutrition and in their application to the problem of the fertilizer requirements of soils. They show the impossibility of making a comparison between the composition of plants of the same species unless the data are referred to the same standard control, i.e., to the composition of the plant of the same species grown under such

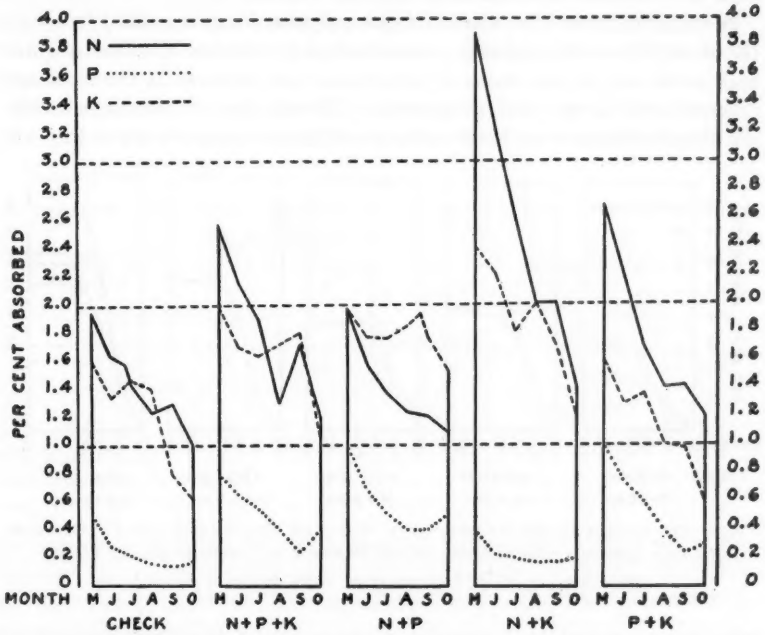


FIG. 3. THE COURSE OF THE ABSORPTION OF NITROGEN, PHOSPHORUS, AND POTASSIUM BY *Vinis vitifera* AS DETERMINED BY THE PERIODIC ANALYSIS OF THE LEAVES
Results are expressed in percentages of dry weight of material

conditions of physiological balance as to produce sufficient growth to ensure reproduction of the highest order.

It is important to ascertain the causes of these apparently anomalous results. But before attempting an explanation the results of some experiments in Austria on barley (75) bearing on this same problem will be given.

The Austrian field experiments.—Both nitrogen and phosphorus in the soil of this experiment are very low but the potassium content is high. The results, which are given in absolute amounts of the element absorbed (fig. 4), show that relative to the absorption of elements from the (N + P + K) plot the

omission of potassium resulted in an increased absorption of phosphorus and nitrogen and that relative to the (2N + P + K) plot the reduction in nitrogen (N + P + K) resulted in a decreased absorption of potassium and phosphorus.

In the unfertilized plot potassium, as would be expected from Liebig's law, is absorbed in relatively greater amounts than nitrogen or phosphorus up to the time of blossoming. The analyses of his plants, however, showed that after this period potassium migrated again to the soil. Sekera maintains that this migration is a physiological necessity and represents the plant's attempt to rid itself of an element absorbed in excess of that utilized.

It will be observed that when nitrogen and phosphorus are added (N + P) the absorption of the elements is increased and potassium does not migrate back to the soil, for the excess of potassium is now balanced by the increased absorption of nitrogen and phosphorus. The addition of potassium to this soil already abundant in this element, as will be seen from the (N + P + K)

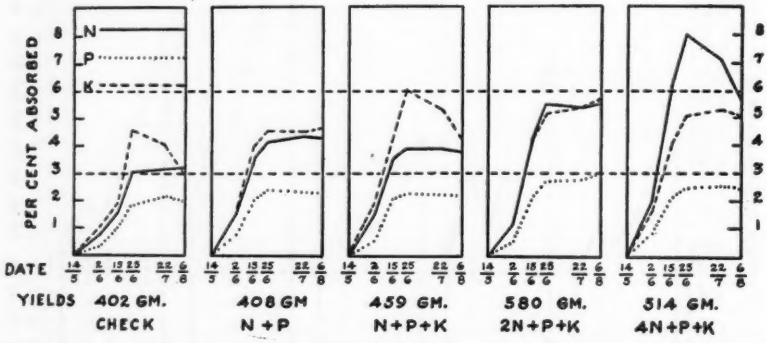


FIG. 4. THE COURSE OF THE ABSORPTION OF NITROGEN, PHOSPHORUS, AND POTASSIUM BY *Hordeum sativum* AS DETERMINED BY THE PERIODIC ANALYSIS OF WHOLE PLANTS
Results are expressed in percentages of dry weight of material

plot, causes a migration of potassium to the soil and a decrease in the amount of nitrogen and phosphorus absorbed. On the further addition of nitrogen, the absorption (2N + P + K plot) again becomes balanced and results in a maximum yield. Further additions of nitrogen result in an excess absorption of this element with migration to the soil and a decreased absorption of potassium and no influence on the absorption of phosphorus.

An analysis and synthesis of the results

Lagatu and Maume (35, 39, 40, 41, 42) have announced that their results clearly show that the corollary from Liebig's "law of the minimum" is not capable of generalization. The present writer will show, however, that the contradiction is an apparent and not a real one.

It may be seen from equation (I) that the effects of the addition of nitrogen,

phosphorus, or potassium to a soil, added singly or in one or more combinations, will—all other factors remaining constant—depend upon the factor $\left(\frac{1}{s_1} + \frac{1}{s_i}\right)$, i.e., (a) upon the supplying power of the soil to maintain concentrations of an element above the critical concentration, and (b) to the concentration with respect to that element produced by the added fertilizer. A knowledge of this supplying power with respect to a given nutrient element would, therefore, appear to be essential in order to determine the ratio between the constituents of a fertilizer necessary to provide a balanced soil solution. Unfortunately, the process of obtaining a true measure of the supplying power with respect to a given nutrient ion is laborious, involving long and expensive experiments (80).

For the purpose of the present analyses, however, sufficient information may be obtained from laboratory methods similar to those of König (33), and of

TABLE 1

Amounts of total solutes and of "available" nitrogen, phosphoric acid, and potash in the soils from the three experiments considered

LOCATION OF EXPERIMENT	DRY SOIL			
	Total solutes	Soluble in 1 per cent K_2SO_4	Soluble in 1 per cent citric acid	
		N	P_2O_5	K_2O
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
State College, Penna, U. S. A.	310–490	82	65	130
Montpellier, France	2,000–2700	260	1,300	210
Vienna, Austria	.....	(low)	47	280

Neubauer (61, 62, 63, 64, 87) and to the displacement (31, 69, 72) or extraction methods (2, 3). Information of this type is available (39, 40, 41, 42, 75, 85) for the check plots of the three experiments discussed. This information is assembled in table 1.

It will be observed that when the soil is deficient in an element, i.e., when s_1 of equation (I) is high $\left(\text{or when } \frac{1}{s_i} \text{ is small}\right)$, the concentration being below the critical level for that element (e.g., nitrogen and phosphorus in the Pennsylvania and Austrian experiments), the omission of the deficient element from the fertilizer has resulted in a *decreased* absorption by the plant of the remaining elements contained in the fertilizer added. Under these conditions the omission of the element from the fertilizer must result in a greater relative deficiency of this element compared with the other two elements and, accordingly, limits (decreases) their absorption as predicated by Liebig's "law."

But when an element is relatively abundant, i.e., when s_1 of equation (*I*) is small, or when $\frac{1}{s_i}$ is large [e.g., nitrogen and phosphorus in the French experi-

ments (34, 36, 37, 38, 39, 40, 41, 42) and potassium in the Austrian experiments (75)], the omission of that element from the fertilizer would not reduce $\frac{1}{s_i}$ of equation (*I*) below the critical concentration for that element. Consequently, the omission of an element already abundant in the soil from a fertilizer results in an *increased* absorption by the plant of the remaining elements added in the fertilizer *relative* to the absorption of these elements from the complete fertilizer plot until the element omitted from the fertilizer becomes a limiting factor, i.e., until its concentration is reduced below the limiting concentration. *The whole problem, therefore, is one of relativity.* In no case is Liebig's law violated.

It should now be apparent that all statements in the literature bearing on the seemingly contradictory results of one worker with those of another on the problem of the mutual action of one element on the solubility of another and of its absorption by plants can be satisfactorily explained in the light of the foregoing interpretation.

The so-called "luxury" consumption of elements

All the experimental evidence (75) appears to indicate that the plant, especially in the early stages of growth up to blossoming, absorbs the nutrient ions in approximately the same proportion that these ions are present in the nutrient medium. Accordingly, if $\frac{1}{s_i}$ or $\left(\frac{1}{s_i}+\frac{1}{s_i'}\right)$ of equation (*I*) is so great as to be out of physiological balance for any element, a "luxuskonsumption" (93) will occur. And, as we have seen, such "luxury consumption" may result in a disturbance to normal metabolism sufficient to affect growth and reproduction (34, 36, 37, 38, 39, 40, 41).

Sekera's experiments (4, 75) which give clear evidence that the excess of an element above that which the plant utilizes in metabolism may be returned to the soil shortly after blossoming occurs have already been discussed. Ions, therefore, may be expelled from the plant in the process of adjustment to more favorable equilibrium conditions. Hoagland's experiments (22, 27) would lead to the view that the mechanism *of this expulsion may be one of exchange of anions or of cations.*

Sekera's experiments (75), moreover, show that within the limits set by the amount of water and solar energy available the return of an element absorbed in excess of that required may be prevented by balancing it through the addition of the other two principal (dominant) nutrient elements (ions), thus enabling the plant to utilize this excess (fig. 4). These experiments, moreover, are of additional interest in indicating a method by means of which the required physiological balance between nitrogen, phosphorus, and potassium may be obtained.

No "luxury" consumption indicated in the old fertility plots of the Pennsylvania State College.—It is of interest to note that results obtained on the absorption

of nitrogen, phosphorus, and potassium by wheat in the old fertility plots having the highest yields of the Pennsylvania State College (56) do not indicate any absorption of an element in excess of that utilized. A comparison of the amounts of these elements absorbed by wheat on plots 17, 19, and 21, receiving increasing amounts of nitrogen as dried blood, shows that the highest application of nitrogen (72 pounds) has not resulted in a lack of balance. The results are given in table 2. We may deduce from this that yields might be further increased by increasing the rate of application of one or more of the carriers of the nutritive elements used in these plots.

TABLE 2

Amounts in pounds per acre applied and recovered in entire wheat crops, of nitrogen, potash, and phosphoric acid on dry basis (after MacIntire)

PLAT NUMBER	TREATMENT	NITROGEN		POTASH		PHOSPHORIC ACID	
		Applied to soil	Removed by plant	Applied to soil	Removed by plant	Applied to soil	Removed by plant
16	6 tons manure	59	34.8	52	37.6	38	18.5
17	(1N)PK	24	29.3	100	42.0	48	17.6
18	8 tons manure	78	39.2	69	36.5	51	19.8
19	(2N)PK	48	37.7	100	44.6	48	20.0
20	10 tons manure	98	44.4	86	46.0	64	22.2
21	(3N)PK	72	45.3	100	61.7	48	24.2
22	6 tons manure + CaO	..	36.4	...	34.9	..	21.6
24	Check	..	18.4	...	16.1	..	9.3

Definition of an optimum fertilizer ratio

Physiological balance within the plant once obtained may again be easily disturbed by the addition of any one of the principal nutrient elements to the soil. *The optimum ratio of the constituents of a fertilizer may then be defined as that ratio which, on the addition of any one of its nutrient constituents—nitrogen, phosphorus, or potassium—results in no increased utilization by the plant of any of the other elements as determined by the time-absorption-graph method.*

The extension of the concept of ionic balance to all elements

The influence of calcium ions.—For the purpose of simplification, the effect of the reciprocal action of elements on absorption has been confined in the present discussion to the three principal nutrients—nitrogen, phosphorus, and potassium—because under the conditions commonly encountered these three elements are the dominant and, indeed, controlling regulatory factors in plant metabolism (71), the functions of which cannot be replaced by any other element. Nevertheless, in practice it is necessary to take cognizance of the effect of other elements and especially of calcium when applications of lime are made regularly. The effect of calcium oxide (or carbonate) on the solubility

of soil nitrogen, phosphorus, and potassium will depend upon the physical and chemical state in which they are present. On the old fertility plots of the Pennsylvania State College CaO and $CaCO_3$ have resulted in depressing the solubility of soil potassium (2, 3). Moreover, this depression in *solubility* is associated, as would be anticipated, with a decreased *absorption* of potassium by the plant (56).

THE MECHANISM OF THE RECIPROCAL ACTION OF IONS IN THEIR RELATION TO ABSORPTION

The nature of the functioning mechanism has not as yet been elucidated. The amount of an ion absorbed in unit time is expressed by the fraction $\frac{1}{sr}$ of equation (*E*), i.e., by the product of the solubility and osmotic value of the respective ions. It is dependent, therefore, upon the structure, chemical composition, and pore space of the root-absorbing surfaces on the one hand, and upon the degree of hydration, atomic volume, and the magnitude of the dissociation constant of the ions, on the other (17, 24, 57, 58, 59, 81, 82).

The relation of dissociation constants to antagonism

The significance of this latter influence is indicated by the experiments of Maume (57, 58, 59) on ion antagonism. In the case of binary electrolytes antagonism is related to the conductivity of the salts after mixture. The antagonistic effect is greatest at that particular concentration of ions represented by the intersection of the dissociation curves of each salt separately determined at dilutions corresponding to the proportions at which each salt is present in the mixture and which is indicated on the abscissa *SS'* (fig. 5). Thus, in the case of NaCl and $CaCl_2$, of all possible proportions of 0.12 Mol. NaCl (soln. *S*) and 0.164 Mol. $CaCl_2$ (soln. S_1) the least toxic mixture is at the point *A* of intersection of the dissociation curves represented by a mixture of 5 cc. $CaCl_2$ + 95 cc. NaCl.

Antagonism is positive.—In the case of binary mixtures of alkali and alkaline earth salts having a common anion the maximum antagonism occurs when the quantity of each salt is such that if separately dissolved in the same volume of water as that of the mixture this would give the same coefficient of ionization. This is indicated by the point represented by the intersection of the curves in figure 5. In this case the common anions will be at different concentrations. For example the molar concentration for NaCl, $CaCl_2$, $Ca(NO_3)_2$, and $NaNO_3$ having the same conductivity are 0.12, 0.22, 0.086, and 0.029, respectively.

Antagonism is nil.—The antagonism is *nil* when, as in the case of the chlorides of the monovalent ions Na^+, K^+ and NH_4^+, for example, the dissociation constant of the mixture is approximately unaltered. This holds when salts having a common ion have, at equal molar concentrations, the same value for the dissociation constant.

Antagonism is negative.—The antagonism is *negative* when, as the result of double decomposition, the degree of dissociation is increased by the formation of different kinds of molecules and ions. Thus:

$$AB + CD = AB + CD + AD + CD + A^+ + B^+ + C^+ + D^+ \ldots\ldots\ldots (J)$$

Causal factors as yet undetermined

These experiments of Maume are of interest in pointing the way to an explanation of the mechanism of antagonism between ions of the *same* electrostatic charge. Nevertheless, because of the present transition stage of our

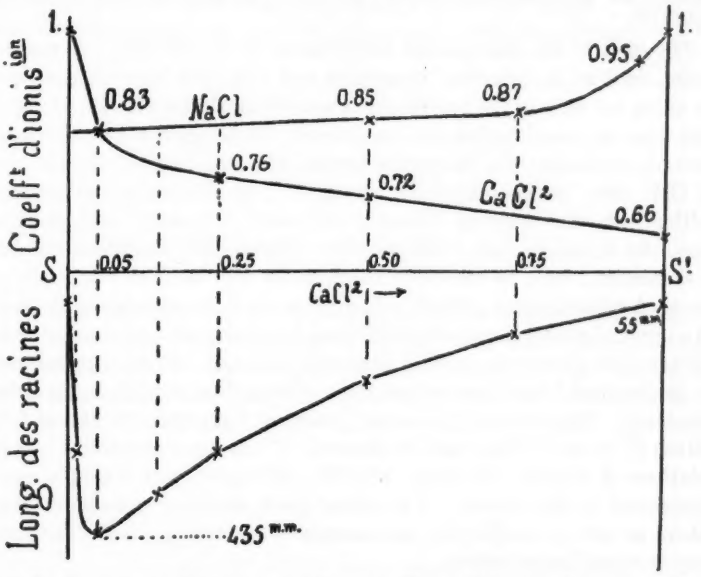


FIG. 5. RELATION OF THE LENGTH OF THE ROOTS OF WHEAT SEEDLINGS GROWING IN SOLUTIONS OF 0.12 MOL. NaCl (SOLUTION *S*) AND OF 0.164 MOL. $CaCl_2$ (SOLUTION S_1) IN THE PROPORTIONS INDICATED TO THE DISSOCIATION CURVES OF THE SEPARATE SALTS DETERMINED AT DILUTIONS CORRESPONDING TO THE PROPORTIONS AT WHICH EACH SALT IS PRESENT IN THE MIXTURE

knowledge concerning the dissociation of strong electrolytes, it would appear that the dissociation constant is only a measure of a causal factor that is as yet obscure. The influence of the motility of ions in relation to dissociation must be considered in determining the actual causal factors operative.

THE INFLUENCE OF OTHER FACTORS IN PRODUCING SELECTIVE ABSORPTION BY PLANTS

The absorption of the so-called physiologically acid and alkali salts

The relation of hydrogen-ion concentration to the absorption of the principal nutrient elements is gradually being elucidated by the application of the rapidly

accumulating facts of physical chemistry. By such means it is possible not only to explain but also to predict the physiological effects of applications of salts such as sodium nitrate and ammonium sulfate.

The rate of absorption of ions under the conditions of hydrogen-ion concentration existent in normal soils (pH 5.5 — 7.0) follows the lyotropic series of Hofmeister (30), viz., for cations the series, in descending order of mobility, is $H > K > Na > Li > Mg > Ba > Sr > Ca$ and for anions $OH > \left.\frac{Bi}{I}\right\} > NO_3 > Cl > HPO_4 > SO_4$ (17). But at hydrogen-ion concentrations outside the aforementioned range the series is irregular and non-predictable (17).

The effect of the hydrogen-ion concentration of the substrate.—A reciprocal action resulting in "selective" absorption may take place between a cation and an anion not only in the presence of a non-diffusible ion (11, 12, 13, 14) but also when no non-diffusible ions are present. If the substrate is slightly acid, neutral, or alkaline, the membrane (roots) will, as a result of the absorption of OH^- ions, become negatively charged. The electrostatic attraction resulting from this potential difference will result in a more rapid absorption (osmosis) of cations than of anions (28). Under these conditions the cation is dominant. But, on the other hand, in an acid medium (especially pronounced below certain critical values *circa* pH 5.5), the membrane (roots), as a result of greater absorption of H^+ ions, becomes positively charged and the electrostatic attraction resulting from this potential difference causes anions to be absorbed faster than cations (28). Under these conditions the anion is dominant. The results of the investigations of Hoagland (23, 26), of Butkewitsch (5, 6) and of Hager and Stollenwerk (17) on the absorption of ions from solutions of $NaNO_3$, $Ca(NO_3)_2$, NH_4OH, $(NH_4)_2SO_4$, and NH_4NO_3 may be interpreted in this manner. *The relative effects, therefore, of these salts may be altered at will by changing the concentration of the hydrogen-ion.* The process may be visualized as follows:

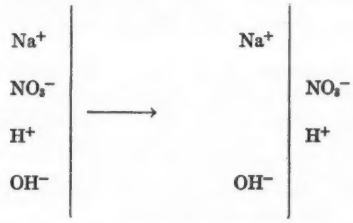
DEGREE OF ACIDITY	CHARGE ON ROOTS	SALT APPLIED
Slightly acid, neutral or alkaline	−	$\underline{NH_4^+} > SO_4^{--}$
	−	$\longleftarrow \underline{Na^+} > NO_3^-$
Distinctly acid, pH 5.5 and below	+	$\longleftarrow \underline{SO_4^{--}} > NH_4^+$
	+	$\longleftarrow \underline{NO_3^-} > Na^+$

where underlining indicates the ion preferentially absorbed, and > indicates the rate of absorption is greater than.

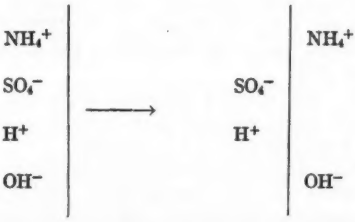
The fact that in alkaline, neutral, or slightly acid media cations exert the dominant influence in absorption, and conversely that in acid media the anions are the dominant factor explains the superiority of ammonium sulfate

over sodium nitrate under the former conditions and of sodium nitrate over ammonium sulfate under the latter. The physiological effect of ammonium salts will accordingly become relatively weaker as the H^+-ion concentration *increases*, whereas the physiological effect of sodium nitrate, on the other hand, will be weaker as the H^+-ion concentration of the soil *decreases*.

The influence of membrane hydrolysis on the "selective" absorption of ions.—It can be shown from thermodynamic principles that an ion cannot permeate the cell membrane without an oppositively charged ion (17). If a dialyzable electrolyte is present on one side of a semi-permeable membrane and on the other pure water, a hydrolytic decomposition of the salt occurs. Using Na^+ as the cation and NO_3^- the anion the process may be illustrated thus:



The median will, therefore, tend to become alkaline because of the much slower rate of penetration of Na^+ compared with NO_3^-. Na^+ is much more strongly hydrated than NO_3^-. On the other hand, if $(NH_4)_2SO_4$ is used the medium will tend to become acid because of the much greater rate of penetration of NH_4^+ compared with SO_4^+. SO_4^- is more strongly hydrated than NH_4^+. Thus:



This hydrolytic action due to the presence of the membrane is promoted in accordance with the Gibbs-Donnan law by the difficultly permeable Na^+ ions in the one case and the SO_4^- ions in the other.

These interpretations are supported by the results of numerous experiments. For example, Prianishnikov (70) found that sugar beet and maize grown in sand cultures, with frequent renewal of the culture solutions so as to maintain practically constant reactions, gave much better results with ammonium salts than with nitrate salts at pH 7.0 but much poorer results with ammonium salts than with nitrate salts at pH 5.5. And Hager and Stollenwerk (17) found

that the ratio of nitrogen to sulfate $\left(\frac{N}{SO_4}\right)$ absorbed from ammonium sulfate by rye, oats, wheat, and barley ranged from 3.06:1 for rye to 5.4:1 for oats.

On the old fertility plots of the Pennsylvania State College the response from ammonium sulfate was better than from sodium nitrate during the first decade but afterwards, as the hydrogen-ion concentration of the soil of the ammonium sulfate plots increased, the response from sodium nitrate was much better than from ammonium sulfate. The pH range of the ammonium sulfate plots is at present (tier I) 4.18–4.50 and of the sodium nitrate plots 5.51–5.71.

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STUDIES ON THE DISPERSION PROCEDURE USED IN THE HYDROMETER METHOD FOR MAKING MECHANICAL ANALYSIS OF SOILS[1]

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In previous communications the hydrometer method has been offered as very rapid, simple, and reasonably accurate for making mechanical analysis of soils. It has been shown (1, 2) that by this method the "total colloid" of soils can be determined in 15 minutes, the conventional clay (0.005–0.000 mm.) in 1 hour, and the fine clay (0.002–0.000 mm.) in 2 hours. In seeking an explanation for this extreme rapidity, which is contrary to Stokes' law, the question has arisen whether it may not be due to an incomplete dispersion of the soils, especially in view of the fact that the soils are dispersed for only about 10 minutes by the mechanical stirrer.

To answer this question certain special tests have been made; they are reported here.

DISPERSING SOILS WITH MECHANICAL STIRRER AND SHAKER

With the exception of the sands and the soils not easily dispersed, the hydrometer method calls for stirring the soils for about 10 minutes. The U. S. Bureau of Soils allows (3) the soils to be shaken for 16 hours for the purpose of mechanical analysis. Comparisons between mechanical analyses of soils which had been stirred 10 minutes with the mechanical stirrer and those of the same soils after 16 hours' shaking with the standard horizontal shaker are reported in table 1.

Table 1 shows clearly that stirring the soils 10 minutes with the mechanical stirrer produces about the same degree of dispersion as shaking them 16 hours with the shaker. With the exception of Fargo clay surface soil and some of the sandy soils, the differences are small and mainly within the limits of experimental error. The larger variation in the Fargo clay surface soil is probably due to the undecomposed organic matter, which on standing a longer time in water undergoes more dispersion in the shaker. The variations in the sands are due to the tendency of the mechanical stirrer to break down the sand particles on long stirring.

[1] Journal Article No. 73 (n.s.) from the Michigan Agricultural Experiment Station.

TREATMENT WITH HYDROGEN PEROXIDE

The international pipette (3) method for making mechanical analyses of soils calls for treating them with hydrogen peroxide, whereas the hydrometer method has not called for such a treatment.

TABLE 1

Comparison of mechanical analyses of soils which were stirred 10 minutes with dispersing machine and those shaken 16 hours with shaker

	STIRRED 10 MINUTES			SHAKEN 16 HOURS		
	Colloid	Clay, 0.005–0.000 mm.	Fine clay, 0.002–0.000 mm.	Colloid	Clay, 0.005–0.000 mm.	Fine clay, 0.002–0.000 mm.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Plainfield sand 0–6 inches	10.7	8.8	6.9	9.5	7.1	6.0
Plainfield sand 6–18 inches	9.6	7.0	5.5	8.4	6.0	4.6
Miles fine sandy loam	10.7	8.8	8.2	9.0	7.2	7.4
Kirven sandy loam	15.7	10.8	9.9	14.0	9.3	8.3
Beardon fine sandy loam, surface	19.6	16.8	13.7	21.6	19.7	17.6
Amarillo fine sandy loam	24.0	19.0	17.0	22.8	18.2	16.0
Loess soil 0–10 inches	25.6	14.5	12.9	26.4	13.8	12.5
Colby silt loam, surface	35.5	22.1	19.1	36.3	23.2	20.8
Colby silt loam, subsoil	35.0	25.5	19.0	36.2	26.0	22.1
Barnes loam	41.1	32.2	29.2	41.8	33.6	30.4
Cecil clay loam, subsoil, unlimed	42.2	37.7	36.5	41.8	38.1	35.3
Cecil clay loam, subsoil, limed	44.3	40.2	38.7	43.3	38.7	36.7
Ava silt loam	42.0	33.6	30.7	42.1	32.9	30.2
Grundy silt loam, subsoil	49.0	36.4	34.0	48.6	35.7	33.6
Lake Charles clay, surface	50.2	42.4	38.9	50.7	42.7	39.8
Lake Charles clay, subsoil	56.2	46.0	42.2	57.6	47.8	43.6
Aberdeen clay 4–10 inches	52.3	45.4	42.9	51.7	44.8	42.5
Welland clay, surface	59.8	49.5	42.5	60.0	51.2	43.1
Welland clay, subsoil	73.0	63.7	56.5	73.8	63.5	57.1
Fargo clay, surface	62.0	45.2	38.9	63.7	53.0	43.9
Fargo clay, subsoil	73.4	59.8	53.6	75.2	62.6	55.1
Osage clay	76.2	61.5	56.8	74.2	62.7	57.3
Parson silt loam B	75.0	64.3	60.1	74.7	65.1	59.2
Parson silt loam hardpan 24 inches	72.2	67.5	67.3	74.1	67.1	63.9
Kindersley clay 1–3 inches	77.6	75.9	72.2	76.9	74.4	72.8
Kindersley clay 3–8 inches	78.2	75.0	71.7	79.4	75.6	72.5
Sharkley clay	83.6	74.7	69.3	82.0	73.7	66.9
Haldemand clay, subsoil	88.4	85.6	77.4	87.1	84.3	76.5

To ascertain the extent to which treatment with hydrogen perioxide effects the results obtained by the hydrometer method, soil samples were treated first with dilute and then with 30 per cent hydrogen peroxide and allowed to stand in a temperature of 65°C. for about 24 hours, with occasional stirring. The soils were then poured into the dispersing cup, stirred, and their mechanical

analyses made by the hydrometer method. For comparison, check samples of the same soils were similarly analyzed by the hydrometer method, but without treatment. The comparative results obtained are shown in table 2.

The results in table 2 show that out of 28 soils studied, only in 2 did the treatment with hydrogen peroxide make a noticeable difference in their me-

TABLE 2

Comparison of mechanical analyses of soils that did with those that did not receive peroxide treatment; both sets of soils being dispersed 10 minutes with the mechanical stirrer

	NOT TREATED			TREATED WITH HYDROGEN PEROXIDE		
	Colloids	Clay, 0.005–0.000 mm.	Fine clay, 0.002–0.000 mm.	Colloids	Clay, 0.005–0.000 mm.	Fine clay, 0.002–0.000 mm.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Plainfield sand 0–6 inches	10.7	8.8	6.9	11.2	8.6	7.2
Plainfield sand 6–18 inches	9.6	7.0	5.5	9.8	7.8	6.3
Miles fine sandy loam	10.7	8.8	8.2	10.3	8.5	9.0
Kirven sandy loam	15.7	10.8	9.9	15.3	11.1	9.5
Beardon fine sandy loam, surface	19.6	16.8	13.7	21.8	19.7	18.6
Amarillo fine sandy loam	24.0	19.0	17.0	26.2	21.2	18.9
Loess soil 0–10 inches	25.6	14.5	12.9	27.0	18.6	16.0
Colby silt loam, surface	35.5	22.1	19.1	35.8	22.8	20.3
Colby silt loam, subsoil	35.0	25.5	19.0	36.2	25.1	21.1
Barnes loam	41.1	32.2	29.2	43.0	32.8	30.5
Cecil clay loam, subsoil, unlimed	42.2	37.7	36.5	43.5	38.1	35.7
Cecil clay loam, subsoil, limed	44.3	40.2	38.7	42.8	38.9	36.7
Ava silt loam	42.0	33.6	30.7	42.5	34.6	31.1
Grundy silt loam, subsoil	49.0	36.4	34.0	49.5	35.8	33.8
Lake Charles clay, surface	50.2	42.4	38.9	51.8	43.5	40.1
Lake Charles clay, subsoil	56.2	46.0	42.2	59.7	47.4	44.5
Aberdeen clay 4–10 inches	52.3	45.4	42.9	52.8	44.7	42.3
Welland clay, surface	59.8	49.5	42.5	61.2	52.0	43.8
Welland clay, subsoil	73.0	63.7	56.5	72.8	64.1	57.0
Fargo clay, surface	62.0	45.2	38.9	69.8	57.9	51.4
Fargo clay, subsoil	73.4	59.8	53.6	76.6	66.8	58.3
Osage clay	76.2	61.5	56.8	74.2	61.7	56.7
Parsons silt loam B	75.0	64.3	60.1	75.7	65.6	59.8
Parsons silt loam hardpan 24 inches	72.2	67.5	67.3	73.6	66.3	63.2
Kindersley clay 1–3 inches	77.6	75.9	72.2	77.4	76.3	71.7
Kindersley clay 3–8 inches	78.2	75.0	71.7	79.4	75.6	72.5
Sharkley clay	83.6	74.7	69.3	82.0	73.7	68.5
Haldemand clay, subsoil	88.4	85.6	77.4	89.1	82.7	76.2

chanical analyses. In all the other soils the treatment made hardly any difference; the small variations that are observed are within the limits of experimental error. The two soils in which the hydrogen peroxide affected the mechanical analysis are Beardon sandy loam and Fargo clay surface, both of

which contain a very large amount of organic matter. From the data obtained with these two soils it would be logically expected that Lake Charles clay both surface and subsoil, Fargo clay subsoil, and Osage clay, all of which contain appreciable quantities of organic matter, would give similar results, but they did not. The probable explanation for this dissimilarity is that the former soils contain a larger amount of organic matter and in a more undecomposed state than the latter soils.

The indications are, therefore, that hydrogen peroxide treatment makes practically no difference in the dispersion of mineral soils containing no, or small amounts of, well-decomposed organic matter, but it makes considerable difference in soils that contain very large amounts of organic matter in an incompletely decomposed state.

STIRRING SOILS FOR DIFFERENT PERIODS WITH MECHANICAL STIRRER

The present procedure of the hydrometer method calls for stirring sands and extreme sandy loams for 5 minutes; loams, silt loams, clay loams, and clays, 10 minutes; and soils containing large amounts of undecomposed organic matter or soils that are recognized to be not easily dispersible, such as some of the lateritic soils and some claypans, 20 minutes or longer.

To measure the effect of long periods of stirring on the mechanical analyses of soils of various types, numerous samples were stirred for 10-minute and 60-minute periods and their mechanical analyses studied by the hydrometer method. The data are presented in table 3.

In general the data in table 3 show that stirring soils 60 minutes tends to increase their apparent content of finer material as compared with that at the 10-minute stirring. The increase, however, is most appreciable in the sands and sandy loams, and in some soils containing large amount of organic matter. In the clay loams and clays the increase is small, and in many cases can well be considered as being within the limits of experimental error.

With few possible exceptions, the increase in the finer material on long stirring is due almost entirely to the breaking up of the sand paricles. The speed of the stirrer (over 10,000 r.p.m.) together with the presence of wire baffles in the cup apparently tends to break up the sand particles. This view is supported by the greater relative increase in the fine material in the sandy soils, as compared with the clays, and also by the results in table 1, wherein it is shown that shaking sands for 16 hours has somewhat less dispersing effect on them than stirring them 10 minutes. It is on account of this possibility of breaking up the sand particles, that the hydrometer procedure (2) calls for stirring sandy soils only about 5 minutes.

The results in table 3 tend to indicate, therefore, that the period of stirring from about 5 to 10 minutes effects almost a complete dispersion of the great majority of soils. Undoubtedly there are some types of soils, such as the laterites, some claypans, and those containing large amounts of organic matter, especially of the undecomposed type, which require more than 10 minutes for

complete dispersion. The studies presented herein, therefore, tend to show that the rapidity with which the hydrometer method measures the mechanical analyses of soils is not due to their incomplete dispersion.

The final procedure of the hydrometer method for determining total colloids in 15 minutes, clay (0.005–0.000 mm.) in 1 hour, and fine clay (0.002–0.000 mm.) in 2 hours, remains the same as previously published (2), except that soils containing very large amounts of organic matter especially of the undecomposed type should be treated with hydrogen peroxide. The further researches and comparisons of the hydrometer method have not caused any

TABLE 3

Comparison of mechanical analyses of soils dispersed by mechanical stirrer for 10-minute and 60-minute periods

	DISPERSED FOR 10 MINUTES			DISPERSED FOR 60 MINUTES		
	Colloids	Clay, 0.005–0.000 mm.	Fine clay, 0.002–0.000 mm.	Colloids	Clay, 0.005–0.000 mm.	Fine clay, 0.002–0.000 mm.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Plainfield sand 0–6 inches	10.7	8.8	6.9	19.0	13.4	11.5
Plainfield sand 6–18 inches	9.6	7.0	5.5	14.1	11.9	9.8
Miles fine sandy loam	10.7	8.8	8.2	13.7	11.7	10.0
Kirven sandy loam	15.7	10.8	9.9	18.5	13.7	12.1
Beardon fine sandy loam, surface	19.6	16.8	13.7	24.4	21.9	20.3
Amarillo fine sandy loam	24.0	19.0	17.0	29.5	26.0	22.4
Loess soil 0–10 inches	25.6	14.5	12.9	30.1	19.6	16.4
Colby silt loam, surface	35.5	22.1	19.1	37.8	26.0	24.5
Colby silt loam, subsoil	35.0	25.5	19.0	37.5	28.2	24.1
Barnes loam	41.1	32.2	29.2	46.1	36.9	32.5
Cecil clay loam, subsoil, unlimed	42.2	37.7	36.5	44.8	39.2	36.8
Cecil clay loam, subsoil, limed	44.3	40.2	38.7	45.9	41.0	39.2
Ava silt loam	42.0	33.6	30.7	44.9	35.3	32.6
Grundy silt loam subsoil	49.0	36.4	34.0	51.3	40.8	38.0
Lake Charles clay, surface	50.2	42.4	38.9	51.3	44.1	41.6
Lake Charles clay, subsoil	56.2	46.0	42.1	58.6	47.3	43.2
Aberdeen clay 4–10 inches	52.3	45.4	42.9	53.7	48.8	45.5
Welland clay, surface	59.8	49.5	42.5	64.8	52.4	48.3
Welland clay, subsoil	73.0	63.7	56.5	73.9	65.8	59.8
Fargo clay, surface	62.0	45.2	38.9	70.3	57.4	51.2
Fargo clay, subsoil	73.4	59.8	53.6	77.4	68.0	59.9
Osage clay	76.2	61.5	56.8	77.3	63.5	57.3
Parsons silt loam B	75.0	64.3	60.1	75.5	67.5	62.7
Parsons silt loam hardpan 24 inches	72.2	67.5	67.3	75.5	68.7	66.8
Kindersley clay 1–3 inches	77.6	75.9	72.2	80.3	76.7	73.4
Kindersley clay 3–8 inches	78.2	75.0	71.7	81.2	76.7	73.2
Sharkley clay	83.6	74.7	69.3	85.5	77.4	71.7
Haldemand clay, subsoil	88.4	85.6	77.4	91.0	84.8	77.1

change in the original finding that the method is reasonably accurate and reliable for all practical purposes, and for the average person it is probably more reliable than any of the present methods.

SUMMARY

The hydrometer method of making mechanical analyses of soils was further investigated by comparing its mechanical stirrer with the standard shaker for dispersing soils. It was found that 10 minutes' stirring with the mechanical stirrer produced about the same degree of dispersion as 16 hours' shaking with the standard shaker.

Treating soils with hydrogen peroxide did not effect their mechanical analysis as measured by the hydrometer method except in soils that contain very large amounts of organic matter, especially of the undecomposed type.

On account of its efficiency, the mechanical stirrer tends to break down the sand particles on long stirring; hence it is advisable not to stir the sandy soils too long.

The extreme rapidity with which the hydrometer method is able to measure the mechanical analysis of soils is not due, therefore, to their incomplete dispersion.

Further research and comparison tend to support the original findings that, for all practical purposes, the hydrometer method is reasonably accurate and reliable.

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FURTHER STUDIES ON THE RELATIONSHIPS BETWEEN THE FINE MATERIAL OF SOILS AND THEIR PHYSICAL CHARACTERISTICS

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In previous communications (5) it was shown that certain soil characteristics, such as heat of wetting, moisture equivalent, and unfree water, bear a close relationship to that portion of the soil which is determined by the hydrometer method (2) at the end of 15 minutes' standing and which has been designated as "total soil colloids." On account of this close relationship it was revealed that these soil characteristics could be indirectly determined very quickly and simply by means of the hydrometer method.

In view of this promising study it was decided to investigate other soil characteristics and the relationship that they bear to the "total soil colloids" and to the conventional clay. One of the soil characteristics that was selected for detailed investigation is the consistency of soils. This characteristic or property of soils must be regarded of considerable importance, judging from the wide attention that it has received of late. It was thought that if the consistencies of soils bear a close relationship to the "total soil colloids" and to the clay as determined by the hydrometer method, then these consistencies could be indirectly determined very quickly and simply by the hydrometer method. It is apparent that this indirect determination would have decided advantage over the direct determination for the following reasons: (*a*) it is exceedingly rapid, (*b*) it is very simple, (*c*) it eliminates the personal element, and (*d*) it might be more reliable because the hydrometer method gives rather accurate results.

As is readily realized, any number of consistencies can be obtained in soils, especially the clays, by varying the moisture content. After preliminary examination and due consideration, three different consistencies were chosen for critical investigation; namely, the crumbling point, the flowing point, and the upper plastic limit of Atterberg (1). It is the object of this paper, therefore, to present the results obtained from the study of these consistencies. They will be considered separately. Further studies on moisture equivalent will also be incidently presented.

CRUMBLING POINT

The crumbling point represents that stage of moisture content at which the soil particle or crumbs have practically no cohesiveness—adhesiveness and

crumble under slight pressure. This is the state at which soils under field conditions would work the easiest—they would plough easily and break to crumbs most readily and not stick to the implements. The moisture content at this crumbling point seems to be at or very close to the wilting coefficient of soils.

The procedure of determining this crumbling point of soils consists of placing about 20 gm. of air-dry soil that has passed through a 2-mm. sieve, into a porcelain dish, adding sufficient distilled water to wet it, and then allowing it to lose water at room temperature. When the soil loses enough water so that it approaches somewhat the crumbling point, it is transferred to a paper on the table and rubbed with the palm of the hand in a circulatory motion. If the soil is near its crumbling point, it is easily reduced to crumbs or compound particles by this rubbing process. Then a small amount of soil is taken and rolled between the fingers and then this rolled piece of soil is taken end by end and pressed between the fingers. If the soil has reached the proper moisture content the rolled piece will readily crumble into crumbs without showing any stickiness or cohesiveness. This is the approximately true crumbling point. If the soil has not reached the proper moisture content the crumbs will tend to stick and do not fall apart easily. In this case, the soil is allowed to lose more moisture as it is being rubbed and mixed on the paper, and is tested often to see if the proper moisture content is reached.

The procedure is of course emperical and would seem to involve considerable personal element, but the transitional point is sufficiently definite to yield closely reproducible results. With little experience one can get good agreements. Extensive tests showed that for the majority of soils results could be reproduced or checked, even at different dates, within 2 per cent.

In table 1 are reported the crumbling points of a fairly large number of representative types of soils coming from many regions.[1] In the same table are also reported the mechanical analysis of "total colloids," clay, and fine clay, as obtained by the hydrometer method (4) and the relationships that these soil fractions bear to the crumbling point.

The last three columns of table 1 readily show that there is a close relationship between the total colloid, clay, fine clay, and the crumbling point. The coefficient of correlation is 0.966 ± 0.013 for the "total colloid," 0.943 ± 0.021 for the clay, and 0.922 ± 0.029 for the fine clay. Reducing these coefficients of correlation to percentages by the process of squaring the R-values and then multiplying by 100, shows how much of the variability in crumbling point is caused by each soil fraction. These calculations show that the variability in crumbling point is influenced 93.8 per cent by the total colloids, 88.9

[1] The writer is grateful for soil samples received to: C. F. Marbut—U. S. Bureau of Soils, E. A. Norton—University of Illinois, A. H. Joel—University of Saskatchewan, A. S. Cline—North Carolina Agricultural College, E. B. Reynolds—Texas Agricultural College, and G. N. Ruhnke—Ontario Agricultural College.

per cent by the clay, and 85.0 per cent by the fine clay. Evidently the crumbling point of soils is almost entirely controlled by their fine material. The

TABLE 1

Relationship between crumbling point of soils, and their content of total colloids, clay, and fine clay

	TOTAL COLLOIDS	CLAY, 0.005–0.000 mm.	FINE CLAY, 0.002–0.000 mm.	CRUMBLING POINT	RATIO CRUMBLING POINT / TOTAL COLLOIDS	RATIO CRUMBLING POINT / CLAY	RATIO CRUMBLING POINT / FINE CLAY
	per cent	*per cent*	*per cent*	*per cent*			
Amarillo fine sandy loam 7–18 inches	24.0	19.0	17.0	6.5	0.2708	0.3421	0.3823
Loess soil 0–10 inches	25.6	14.5	13.0	7.4	0.2891	0.5103	0.5692
Stewart sandy loam B	28.6	20.5	14.0	7.4	0.2587	0.3609	0.5285
Colby silt loam, surface	35.0	22.1	19.1	10.0	0.2857	0.4524	0.5235
Colby silt loam, subsoil	35.0	25.5	19.0	9.2	0.2628	0.3608	0.4842
Barnes loam 20–40 inches	41.1	32.2	29.2	11.5	0.2798	0.3571	0.3938
Ava silt loam, subsoil	42.0	33.6	30.7	10.9	0.2595	0.3244	0.3550
Cecil clay loam, subsoil, unlimed	42.2	37.7	36.5	11.5	0.2725	0.3052	0.3151
Cecil clay loam, subsoil, limed	44.3	40.2	38.7	11.8	0.2655	0.2935	0.3050
Davison loam 0–9 inches	42.4	35.9	31.0	11.4	0.2688	0.3175	0.3677
Marshall silt loam 4–18 inches	45.3	35.6	31.4	13.2	0.2913	0.3707	0.4204
Grundy silt loam, subsoil	49.0	36.5	34.0	14.0	0.2857	0.3861	0.4117
Lake Charles clay, surface	50.2	42.4	38.9	13.4	0.2669	0.3160	0.3444
Lake Charles clay, subsoil	56.2	46.0	42.1	13.1	0.2331	0.2847	0.3111
Soil from Fallon, Nevada	49.9	40.6	36.3	13.9	0.2785	0.3424	0.3824
Aberdeen clay 4–10 inches	52.3	45.4	42.9	12.2	0.2332	0.2687	0.2843
Welland clay, surface	59.8	49.5	42.5	17.7	0.2959	0.3575	0.4164
Welland clay, subsoil	73.0	63.7	56.5	20.8	0.2849	0.3265	0.3681
Fargo clay, surface	69.6	57.9	51.4	21.0	0.3017	0.3626	0.4085
Fargo clay, subsoil	76.6	66.8	58.2	20.6	0.2689	0.3084	0.3539
Kinderly clay 1–3 inches	77.6	75.9	72.2	19.3	0.2487	0.2543	0.2673
Kinderly clay 3–8 inches	78.2	75.0	71.7	19.9	0.2544	0.2653	0.2775
Parsons silt loam B	75.0	64.3	60.1	17.9	0.2386	0.2783	0.2978
Osage clay	76.2	61.5	56.8	18.2	0.2388	0.2959	0.3204
Sharkley clay	83.6	74.7	69.3	20.5	0.2452	0.2744	0.2958
Miami silty clay loam 14–18 inches	80.0	68.3	63.0	19.0	0.2437	0.2855	0.3095
Haldemand clay, subsoil	88.4	85.6	77.4	20.6	0.2335	0.2412	0.2667
Average ratio					0.2617	0.3092	0.3048
Coefficient of correlation					0.966	0.943	0.922
Coefficient of correlation reduced to percentage basis					93.3	88.9	85.0
Probable error					0.013	0.021	0.029

influence exerted by other factors such as the nature of the fine soil material is comparatively small.

In view of these close relationships the crumbling point of any soil can be

determined indirectly and quickly by means of the hydrometer method. This can be accomplished according to the following formulas:

$$\text{Average } \frac{\dfrac{\text{Total colloids}}{\text{Crumbling point}}}{\text{Total colloids}} \text{ Ratio} = \text{Crumbling point}$$

$$\text{Average } \frac{\dfrac{\text{Clay}}{\text{Crumbling point}}}{\text{Clay}} \text{ Ratio} = \text{Crumbling point}$$

$$\text{Average } \frac{\dfrac{\text{Fine clay}}{\text{Crumbling point}}}{\text{Fine clay}} \text{ Ratio} = \text{Crumbling point}$$

Since the "total colloids" formula involves only 15 minutes and it seems to give results just as good as, or better than, the clay and fine clay formulas which require 1 and 2 hours respectively, then the "total colloid" formula can be preferably used to determine the crumbling point of soils.

FLOWING POINT

The flowing point was chosen to represent one of the extremes toward the wet side of the soil as the crumbling point was selected to represent one of the extremes on the dry side.

In order to reduce the results to a comparable and uniform basis the soils were subjected to dispersion.

The final procedure adopted for measuring the flowing point of soils consists of placing about 50 gm. of soil into a 100-cc. beaker, adding sufficient distilled water to saturate it, and then mixing it and dispersing it by means of the motor shown in plate 1. The dispersion is continued with the addition of small amounts of water until the mixture commences to flow, when the beaker is turned to an angle and the mixture does not thicken on further dispersion.

The soil is dispersed for at least 15 minutes. The more the soil is dispersed the more water it requires to come to the flowing point. When it is about completely dispersed, additional stirring does not change its consistency or flowing point.

The process of dispersion tends to increase greatly the amount of water that many clay soils will take up to reach the flowing point. In some instances it is almost 100 per cent increase, whereas in other soils the increase is small.

In table 2 are presented the results on flowing point of the same soils as used in the crumbling point studies. In the same table is shown the relationship that exists between the flowing point of the various soils and their "total colloid," clay, and fine clay.

The results in table 2 show that there is a good correlation between the flowing point and the "total colloid," clay, and fine clay of the various soils.

The coefficient of correlation for all of the soils is .921 ± .029 for the "total colloid," .906 ± .034 for the clay, and .915 ± .031 for the fine clay. When these figures are expressed on a percentage basis it is seen that the variability

TABLE 2

Relationships between the finer material of soils and their flowing points*

	FLOWING POINT	RATIO F. P. / TOTAL COLLOIDS	RATIO F. P. / CLAY	RATIO F. P. / FINE CLAY
	per cent			
Amarillo fine sandy loam 7–18 inches	57.3	2.3875	3.0157	3.3705
Loess soil 0–10 inches	41.9	1.6367	2.8896	3.2230
Stewart sandy loam B	38.4	1.3426	1.8731	2.7428
Colby silt loam, surface	50.1	1.4314	2.2669	2.6231
Colby silt loam, subsoil	55.5	1.5857	2.1764	2.9210
Barnes loam 20–40 inches	72.0	1.7516	2.2360	2.4657
Ava silt loam, subsoil	81.0	1.9285	2.4107	2.6384
Cecil clay loam, subsoil, unlimed	65.3	1.5473	1.7320	1.7890
Cecil clay loam, subsoil, limed	65.3	1.4740	1.6243	1.6873
Davison loam 0–9 inches	77.5	1.8278	2.1587	2.5000
Marshall silty loam 14–18 inches	93.4	2.0618	2.6235	2.9745
Grundy silt loam, subsoil	90.0	1.8367	2.4724	2.6239
Lake Charles clay, surface	89.3	1.7788	2.1061	2.2956
Lake Charles clay, subsoil	101.8	1.8113	2.2130	2.4181
Soil from Fallon, Nevada	88.4	1.7715	2.1773	2.4352
Aberdeen clay 4–10 inches	93.1	1.7801	2.0506	2.1701
Welland clay, surface	81.5	1.3628	1.6464	1.9176
Welland clay, subsoil	120.0	1.6438	1.8838	2.1238
Fargo clay, surface	81.0	1.164	1.3989	1.4336
Fargo clay, subsoil	106.8	1.4550	1.7859	1.9925
Kinderley clay 1–3 inches	130.5	1.6817	1.7193	1.8074
Kinderley clay 3–8 inches	118.0	1.5089	1.5733	1.6457
Parsons silt loam B	126.2	1.6826	1.9626	2.0998
Osage clay	107.0	1.4041	1.7398	1.8838
Sharkley clay	128.8	1.5406	1.7242	1.8585
Miami silty clay loam 14–18 inches	104.0	1.3870	1.5226	1.6507
Haldemand clay subsoil	134.2	1.5180	1.5677	1.7338
Average ratio		1.6071	1.8977	2.0788
Coefficient of correlation		0.921	0.906	0.915
Coefficient of correlation reduced to percentage basis		84.80	82.10	83.70
Probable error		0.029	0.034	0.031

* Percentages of total colloids of clay 0.005–0.000 mm., and of fine clay 0.002–0.000 mm. are given in table 1.

in the flowing point of soils is influenced about 84.8 per cent by their "total colloids," 82.1 per cent by their clay content, and about 83.7 per cent by their fine clay content.

Although these figures indicate a very good correlation when all the soils are taken as a whole, the ratios shown in the last three columns of the table reveal that in a few cases there is considerable individual variation in the relationship. This occasional individual variation suggests that the property of flowing is not controlled in all soils as completely by the "colloidal" or clay content as are some of the other properties, such as the crumbling point and moisture equivalent.

Probably the chemical composition of a soil would tend to influence the flowing point because some soils of certain composition or with certain salts present would lend themselves to emulsification and others would not. For instance, Parsons silt loam of the C horizon is derived from fuller's earth, and any soil that is derived from fuller's earth or from bentonite tends to emulsify and gelatinize and thereby take up a large amount of water to reach the flowing point. On the other hand, soils such as Cecil loam show practically no emulsification and consequently do not take up as much water to reach the flowing point.

On account of this individual variation of certain soils, it is probably not safe to apply a formula to determine the flowing point for all soils. According to the results in table 2, the flowing point of the vast majority of soils can be determined comparatively accurately by the indirect method shown in the following but for some soils the indirect determination may not be so accurate. The formulas for the indirect determination are as follows:

$$\text{Average}\ \frac{\text{Total colloids}}{\frac{\text{Flowing point}}{\text{Total colloids}}\ \text{Ratio}} = \text{Flowing point}$$

$$\text{Average}\ \frac{\text{Clay}}{\frac{\text{Flowing point}}{\text{Clay}}\ \text{Ratio}} = \text{Flowing point}$$

$$\text{Average}\ \frac{\text{Fine clay}}{\frac{\text{Flowing point}}{\text{Fine clay}}\ \text{Ratio}} = \text{Flowing point}$$

The flowing point appears to possess considerable significance in that it may prove to be very useful in distinguishing and selecting clays for various purposes in the general ceramic industry. In a subsequent paper it will be shown that it can be used to distinguish the various kinds of bentonites.

UPPER PLASTIC LIMIT

The conventional procedure (1) was used to measure the upper plastic limit of the same soils that have been used for the crumbling point and flowing point. The general results are detailed in table 3.

The results in table 3 also show that there is a strong tendency for the "total colloids," clay, and fine clay to control the plasticity of soils. The coefficient

of correlation for all the soils is .916 ± .031 for the "total colloids," .914 ± .032 for the clay, and .920 ± .030 for the fine clay. In other words, the variability of the upper plastic limit of soils seems to be influenced to the extent of

TABLE 3

Relationships between the finer materials of soils and their upper plastic limits*

	UPPER PLASTIC LIMIT	RATIO U. P. L. / TOTAL COLLOIDS	RATIO U. P. L. / CLAY	RATIO U. P. L. / FINE CLAY
	per cent			
Amarillo fine sandy loam 7–18 inches	18.6	0.7750	0.9789	1.0947
Loess soil 0–10 inches	22.0	0.8590	1.5170	1.7100
Stewart sandy loam B	17.1	0.5980	0.8340	1.222
Colby silt loam, surface	27.7	0.7914	1.2533	1.4502
Colby silt loam, subsoil	22.5	0.6428	0.8823	1.1842
Barnes loam 20–40 inches	27.3	0.6645	0.8480	0.9350
Ava silt loam, subsoil	30.7	0.7309	0.9136	1.0000
Cecil clay loam, subsoil, unlimed	26.5	0.6279	0.7029	0.7260
Cecil clay loam, subsoil, limed	25.4	0.5733	0.6318	0.6563
Davison loam 0–9 inches	29.7	0.7004	0.8272	0.9580
Marshall silt loam 14–18 inches	40.7	0.8984	1.1432	1.2961
Grundy silt loam, subsoil	27.7	0.5653	0.7909	0.8076
Lake Charles clay, surface	39.6	0.7888	0.9339	1.0179
Lake Charles clay, subsoil	45.9	0.8167	0.9978	1.0902
Soil from Fallon, Nevada	29.5	0.5911	0.7266	0.8127
Aberdeen clay 4–10 inches	43.9	0.8393	0.9669	1.0233
Welland clay, surface	40.3	0.6740	0.8141	0.9482
Welland clay, subsoil	43.2	0.5920	0.6782	0.7645
Fargo clay, surface	48.9	0.7025	0.8455	0.8655
Fargo clay, subsoil	49.3	0.6716	0.8244	0.9198
Kindersley clay 1–3 inches	63.1	0.8131	0.8315	0.8739
Kindersley clay 3–8 inches	64.9	0.8299	0.8653	0.9052
Parsons silt loam B	55.0	0.7333	0.8553	0.9151
Osage clay	47.4	0.6221	0.7707	0.8345
Sharkley clay	61.9	0.7404	0.8286	0.8932
Miami silty clay loam 14–18 inches	46.7	0.6230	0.6837	0.7412
Haldemand clay subsoil	54.8	0.6199	0.6401	0.7080
Average ratio		0.7035	0.8311	0.9104
Coefficient of correlation		0.916	0.914	0.920
Coefficient of correlation reduced to percentage basis		83.90	83.50	84.60
Probable error		0.031	0.032	0.030

* Percentages of total colloids, of clay 0.005–0.000 mm., and of fine clay 0.002–0.000 mm. are given in table 1.

about 83.9 per cent by the total colloids, 83.5 per cent by the clay, and 84.6 per cent by the fine clay.

Although these are good correlations for all the soils as a whole, an examina-

tion of the last three columns shows that some individual soils exhibit considerable variation from the majority.

The formulas for measuring the upper plastic limit of soils indirectly by means of the hydrometer method are as follows:

$$\text{Average } \frac{\text{Total colloids}}{\text{Upper plastic limit}}_{\text{Total colloids}} \text{Ratio} = \text{Upper plastic limit}$$

$$\text{Average } \frac{\text{Clay}}{\text{Upper plastic limit}}_{\text{Clay}} \text{Average ratio} = \text{Upper plastic limit}$$

$$\text{Average } \frac{\text{Fine clay}}{\text{Upper plastic limit}}_{\text{Fine clay}} \text{Average ratio} = \text{Upper plastic limit}$$

MOISTURE EQUIVALENT

The method described in a previous communication (3) was used to measure the moisture equivalent of the same soils that were employed in the preceding studies. In table 4, are presented the general results obtained.

The data in table 4 show good correlations between the moisture equivalent and the "total colloids," clay and fine clay. The correlation for the total colloids is .909 ± .033, for the clay is .895 ± .038, and for the fine clay is .897 ± .038. Reducing these figures to the percentage basis they indicate that the variability of the moisture equivalent is influenced to the extent of about 82.7 per cent by the total colloids, 80.1 per cent by the clay, and 80.5 per cent by the fine clay.

The formulas for determining the moisture equivalent of soils indirectly by means of the hydrometer method are as follows:

$$\text{Colloids} \times \frac{\text{Moisture equivalent}}{\text{Colloids}} \text{Average ratio} = \text{Moisture equivalent}$$

$$\text{Clay} \times \frac{\text{Moisture equivalent}}{\text{Clay}} \text{Average ratio} = \text{Moisture equivalent}$$

$$\text{Fine clay} \times \frac{\text{Moisture equivalent}}{\text{Fine clay}} \text{Average ratio} = \text{Moisture equivalent}$$

In view of the variations that some individual soils show in some of the preceding correlations, it was desired to ascertain whether these variations were due to differences in chemical compositions of the respective soils. Accordingly, the very fine material of several of the soils which showed variations, was analyzed[2] for silica, aluminum, and iron. The fine material analyzed was

[2] The chemical analysis was made by O. B. Winter of this station.

TABLE 4

Relationship between the finer material of soils and their moisture equivalents*

	MOISTURE EQUIVALENT	RATIO M. E. / TOTAL COLLOIDS	RATIO M. E. / CLAY	RATIO M. E. / FINE CLAY
Amarillo fine sandy loam 7–18 inches	19.4	0.8083	1.021	1.1411
Loess soil 0–10 inches	18.4	0.7187	1.268	1.4150
Stewart sandy loam B	16.5	0.5770	0.8050	1.1780
Colby silt loam, surface	24.9	0.7114	1.1266	1.303
Colby silt loam, subsoil	26.2	0.7485	1.027	1.379
Barnes loam 20–40 inches	21.5	0.5231	0.6677	0.7363
Ava silt loam, subsoil	24.0	0.5713	0.7140	0.7765
Cecil clay loam, subsoil, unlimed	24.2	0.5734	0.6419	0.6630
Cecil clay loam, subsoil, limed	24.8	0.5598	0.6169	0.6408
Davison loam 0–9 inches	28.0	0.6603	0.7799	0.9032
Marshall silty loam 14–18 inches	33.0	0.7284	0.9269	1.051
Grundy silt loam, subsoil	35.0	0.7142	0.9615	1.029
Lake Charles clay, surface	39.5	0.7868	0.9316	1.052
Lake Charles clay, subsoil	42.3	0.7526	0.9195	1.005
Soil from Fallon, Nevada	38.5	0.7712	0.9482	1.061
Aberdeen clay 4–10 inches	38.8	0.7418	0.8546	0.9044
Welland clay, surface	36.7	0.6137	0.7414	0.8635
Welland clay, subsoil	39.5	0.5412	0.6200	0.6990
Fargo clay, surface	53.5	0.7686	0.9240	1.0408
Fargo clay, subsoil	42.0	0.5483	0.6287	0.7216
Kindersley clay 1–3 inches	51.4	0.6623	0.6772	0.7119
Kindersley clay 3–8 inches	61.8	0.7902	0.8240	0.8619
Parsons silt loam B	48.5	0.6466	0.7542	0.8069
Osage clay	50.0	0.6561	0.8130	0.8802
Sharkley clay	53.0	0.6339	0.7095	0.7647
Miami silty clay loam 14–18 inches	43.0	0.5374	0.6295	0.6830
Haldemand clay subsoil	48.5	0.5486	0.5665	0.6266
Average ratio		0.6550	0.7734	0.8525
Coefficient of correlation		0.909	0.895	0.897
Coefficient of correlation reduced to percentage basis		0.8270	0.8010	0.8050
Probable error		0.033	0.038	0.038

* Percentages of total colloids, of clay 0.005–0.000 mm., and of fine clay 0.002–0.000 mm. are given in table 1.

TABLE 5

Comparison of physical properties of soils with their chemical compositions

	SiO_2	$Fe_2O_3+Al_2O_3$	Fe_2O_3	Al_2O_3	CRUMBLING POINT	FLOWING POINT	UPPER PLASTIC LIMIT	MOISTURE EQUIVALENT
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Cecil clay loam 7–18 inches	39.2	49.2	12.4	36.8	11.5	65.3	26.5	24.2
Welland clay subsoil	47.84	40.06	13.01	27.05	20.8	120.0	43.2	39.5
Aberdeen clay 4–10 inches	50.65	23.70	7.44	16.26	12.2	93.1	43.9	38.8
Barnes loam	49.68	28.20	6.63	21.57	11.5	72.0	27.3	21.5
Haldemand clay, subsoil	50.50	28.10	8.81	19.29	20.6	134.2	54.8	48.5
Osage clay	52.01	27.50	8.16	19.34	18.2	128.0	47.4	50.0
Kindersley clay 1–3 inches	53.01	24.75	7.71	17.04	19.3	130.5	63.1	51.4
Fargo clay, surface	52.54	20.30	8.08	12.22	13.4	106.8	48.9	53.5
Amarillo fine sandy loam	50.91	30.39	8.31	22.08	6.5	57.3	18.6	19.4

obtained from soil suspension which had stood for 30 hours. In table 5 are shown the chemical compositions of the fine material of a number of soils together with their crumbling point, flowing point, upper plastic limit, and moisture equivalent for comparison.

The data in table 5 reveal that there is no definite relationship between the physical properties of the soils studied and the content of aluminum, silica, and iron of their fine material. The variations in the correlations of individual soils, therefore, cannot be explained on the basis of differences of these constituents in the fine material of these soils. These findings, however, do not contradict the results of other investigators who have found that there is a relationship between certain physical properties of soils as a whole and their chemical composition.

Referring once again to tables 1, 2, 3, and 4, one will see that the "total colloids"—the soil material determined at 15 minutes standing by the hydrometer method—shows a closer correlation with the various soil physical properties studied than does either the conventional clay or the fine clay. These results tend to confirm the views previously expressed that the "total colloids" are more representative of the nature and texture of the soil material that controls the physical properties of soils, than is the clay, fine clay, or true colloids.

Finally it should be emphasized that although the soils employed give a fairly representative list of different types, undoubtedly there may be some types or individual soils which will not show as good correlations between the fine material and physical characteristics as are revealed in the foregoing. The sands, mucks, and peats, for instance, do not show such good correlations, nor would it be expected.

SUMMARY

A study was made to ascertain whether there are close relationships between the "total colloids," clay, and fine clay of soils as determined by the hydrometer method, and certain consistencies of soils such as the crumbling point, flowing point, and upper plastic limit. The idea underlying this investigation was that, if there do exist such close relationships, then the consistencies and other soil physical properties can be indirectly determined very quickly and simply and probably more accurately by means of the hydrometer method.

From the results obtained coefficients of correlation were worked out, and these in turn were reduced to the percentage basis.

The coefficients of correlation on the soils worked show that the variability of the crumbling point is controlled 93.3 per cent by the "total colloids," 88.93 per cent by the clay, and 85.0 per cent by the fine clay.

The variability of the flowing point is controlled 84.8 per cent by the "total colloids," 82.1 per cent by the clay, and 83.7 per cent by the fine clay.

The variability of the upper plastic limit is controlled 83.9 per cent by the "total colloids," 83.5 per cent by the clay, and 84.6 per cent by the fine clay.

The variability of the moisture equivalent is influenced 82.7 per cent by the "total colloids," 80.1 per cent by the clay, and 80.5 per cent by the fine clay.

No definite relationship could be established between the chemical composition of the fine material of soils studied and their physical properties studied.

Undoubtedly there may be some types or individual soils which will not show as good correlation between the fine material and physical characteristics as revealed in soils studied here.

From the correlations obtained it would seem that the hydrometer method can be employed to obtain indirectly and quickly certain physical characteristics of soils.

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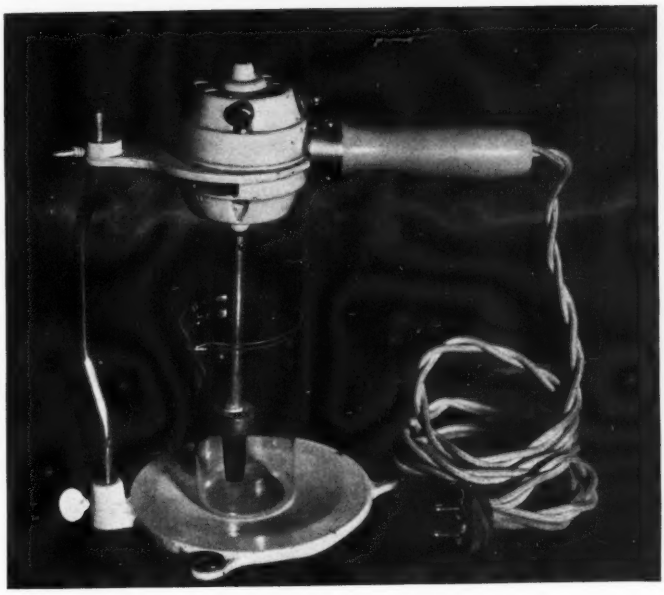
PLATE 1

MACHINE USED TO DISPERSE SOILS IN DETERMINING THE FLOWING POINT OF SOILS



THE LAWS OF SOIL COLLOIDAL BEHAVIOR: VII. PROTEINS AND PROTEINATED COMPLEXES[1]

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Among the inorganic soil colloids we have distinguished between two types of ampholytoids. In the first place we have the amphoteric sesquioxides, which may be classified as primary ampholytoids. Then there is a practically infinite number of amphoteric complexes, derived from the sesquioxides, which constitute insoluble salts with weak acids such as silicic, phosphoric, and humic acids. These might be looked upon as secondary ampholytoids. The law governing their formation and their behavior has been the subject of the last four sections of this series (9, 10, 11, 12).

Among the organic colloids of the soil we meet with a similar grouping of materials. Here the primary ampholytoids are represented by the "pure" proteins whereas the secondary organic ampholytoids constitute an endless number of protein compounds or, what we have chosen to call, proteinated complexes. It is these complexes which form the subject matter of this paper.

Although the isoelectric pH of the hydroxides of Al and Fe [about 8.1 and 7.1 respectively when precipitated from the chlorides by NaOH (9)] is so high that these ampholytoids play their most important rôle in the soil as basoids, the isoelectric pH of many of the proteins is such as to bring out their dual character within the usual range of soil reaction. Michaelis (13) gives 4.7 as the isoelectric pH of the three proteins casein, serum albumin, and gelatin. Edestin, as we shall see, has a higher isoelectric pH, or about 5.2. Edestin is therefore a stronger base and a weaker acid than either of the former proteins. Such differences are probably due to the relative proportions of the various amino acids and their form of linkage in the protein molecules. A protein containing a high proportion of the diamino-mono carboxylic acids arginine and lysine might be expected to be more basic and less acid and to have a higher isoelectric pH than proteins having a low content of these constituents. On the other hand a protein containing a high proportion of the mono-amino di-carboxylic glutamic and aspartic acids might be expected to be more acid and to have a lower isoelectric pH. Thus the protamine salmin, which is reported to contain as high as 88 per cent arginine, possesses an alkaline re-

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

action and would undoubtedly show a very high isoelectric pH, whereas gliadin, which contains as high as 42 per cent glutamic acid, is one of the most acid proteins and is probably isoelectric at a comparatively low pH.

In the present work it would have been highly desirable to study proteins representing a wide variation in their acid and basic properties but unfortunately none but the more common ones; namely, egg albumen, casein, edestin, and gelatin were available. Of these four proteins edestin, however, differs sufficiently in its acid and basic properties and in its isoelectric pH to bring out fundamental differences in amphoteric behavior.

THE BUFFER CAPACITY AND THE ELECTROKINETICS OF THE ("FREE") PROTEINS

The various proteins were first studied as follows: One gram protein was dissolved in a solution containing 1 m. mol NaOH and diluted to 1 liter. Twenty cubic centimeter portions of the protein solutions were mixed with 30 cc. water containing various amounts of HCl. The pH was determined colorimetrically where there was no interference by color reactions. Where this was the case the determinations were made by the quinhydrone electrode. The cataphoresis measurements were made by the ultra-microscopic method as previously described (6).

Gelatin, as is well known, does not coagulate even at the isoelectric point. No gelatin particles were therefore visible in the ultramicroscope. This is also true of the other proteins except at or near the isoelectric point, for these all form an isoelectric floc. Where no particles were visible the technique previously employed (5) was resorted to. This consists in adding a very small amount of an inert powder such as pumice. It appears as if the pumice particles coat themselves with the protein and behave electrokinetically as if they consisted of protein. No difference in cataphoresis was observed when about 10 times the necessary amount of pumice was added. Nor is there a break in the curves of casein, edestin, and albumin near the isoelectric point where no pumice was added. The method is therefore justified both by the results and by the fact that there is at present no other way of directly observing the cataphoretic movement of ultramicroscopically invisible particles. [The method of The Svedberg (14) who photographed the movement of the protein solution by the use of ultraviolet light would be too elaborate where a great number of measurements are to be made.]

The proteins, which were of the kind supplied by chemical firms, had the following composition:

NAME OF PROTEIN	EGG ALBUMIN (EIMER AND AMEND)	CASEIN (HARRIS)	EDESTIN C.P. (EIMER AND AMEND)	BACTO GELATIN ("DIFCO" STANDARD)
Water, per cent, at 105°C.	10.72	8.86	7.66	12.45
Ash, per cent	3.25	3.09	1.06	2.43
N, per cent, 105° basis	14.33	15.67	17.67	18.06

The results of the experiments are shown in table 74 and in figure 11.

The electrokinetic behavior of the four proteins shows significant differences. All, except gelatin, coagulate over a narrow zone at the isoelectric pH. The albumin was isoelectric at pH 4.95, and the casein was, by interpolation, isoelectric at a pH slightly above 4.8. Edestin and gelatin were both found isoelectric at about 5.2.

TABLE 74

Cataphoresis of albumin, casein, edestin, and gelatin (20 cc. of a solution containing 1 gm. protein + 1 millimol NaOH in 1,000 cc.)

0.01 *N* HCl*	ALBUMIN			CASEIN			EDESTIN			GELATIN		
	Floc	μ/sec. 1 volt/cm.	pH	Floc	μ/sec. 1 volt/cm.	pH	Floc	μ/sec. 1 volt/cm.	pH	Floc	μ/sec. 1 volt/cm.	pH
cc.												
0.4	0	−2.75	9.6	+	−2.75	8.4	0	−3.03	8.9	0	−1.38	9.1
0.8	0	−2.63	9.2	+	−2.36	7.1	0	−3.03	8.4	0	−1.01	8.7
1.6	0	−2.52	6.8	+	−2.16	5.9	+	−2.63	6.2	0	−0.67	5.95
2.0	.....	.....	....	.....	.....	....	+++	−1.08	5.6	0	−0.43	5.4
2.2	.....	.....	....	.....	.....	....	++++	−0.76	5.3	0	±0.0	5.2
2.4	.....	.....	....	++	−1.89	5.2	+++	+0.74	5.1	0	+0.42	5.0
2.6	++++	−0.38	5.1	+++	−1.68	5.0	++	+1.51	5.0	0	+0.61	4.7
2.7	++++	±0.0	4.95	.....	.....	....	.....	.....	....	...	.....	....
2.8	++++	+0.20	4.85	++++	−0.61	4.9	++	+1.89	4.95	0	+0.78	4.5
3.0	+++	+0.52	4.75	++++	+0.15	4.8	.....	.....	....	...	.....	....
3.2	0	+0.95	4.60	+++	+0.76	4.7	+	+2.33	4.9	0	+1.21	4.15
3.6	0	+1.42	4.45	++	+1.12	4.45	.....	.....	....	...	.....	....
6.4	0	+2.02	4.2	++	+1.32	4.2	+	+2.52	4.5	0	+1.32	3.4
12.8	0	+2.20	....	+	+2.08	3.8	0	.....	4.3	0	+1.38	2.8
25.6	0	+2.64	....	+	+2.52	3.5	0	+2.75	3.8	0	+1.51	2.4

0 = clear; + = opalescent; ++ = dense opal; +++ = milky; ++++ = flocculation.
* Plus enough water to make 50 cc.

The casein was studied in greater detail. Thus when 2.9 cc. 0.01 *N* HCl was added to the casein solution, in duplicate tubes, the pH was 4.8 and the protein slightly negative. When H_2SO_4 and H_3PO_4 were added to the Na-caseinate instead of HCl the results were as follows:

0.01 *N* H_2SO_4	FLOC	μ/SEC.	pH
cc.			
2.9	++++	−0.24	4.8
3.0	++++	+0.24	4.7
0.01 *M* H_3PO_4			
cc.			
2.9	++++	−0.24	4.8
3.0	++++	+0.16	4.7

This all indicates an isoelectric point between 4.7 and 4.8 and that the casein "sulfate" and "phosphate" are as soluble as the "chloride." The common

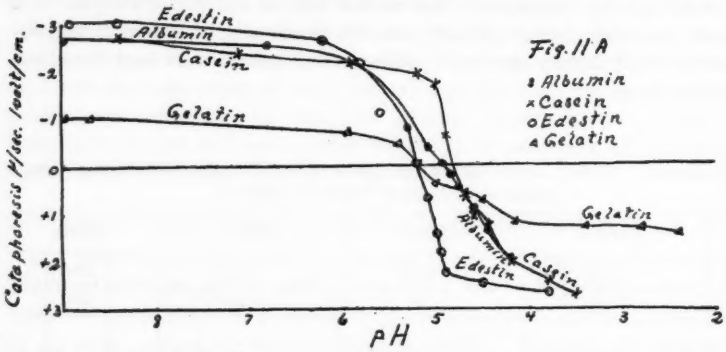


FIG. 11A. CATAPHORESIS OF ALBUMIN, CASEIN, EDESTIN, AND GELATIN AT DIFFERENT pH VALUES, ADJUSTED BY NaOH AND HCl

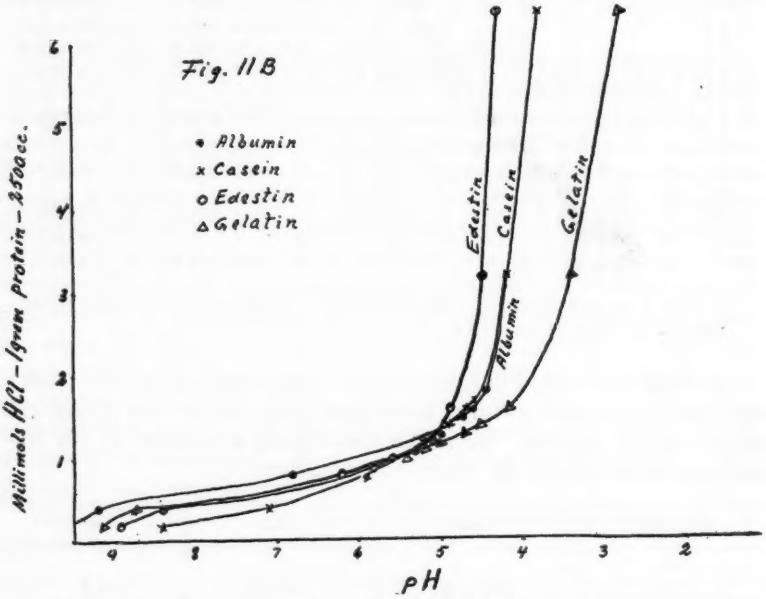


FIG. 11B. THE BUFFER CAPACITY OF EDESTIN, ALBUMIN, CASEIN, AND GELATIN IN HCl SOLUTION

The original solutions contained 1 gm. protein + 1 millimol NaOH in 2,500 cc.

mineral anions do not, therefore, affect the isoelectric point of the proteins. (The isoelectric point of the sesquioxides is, as we have seen, greatly displaced

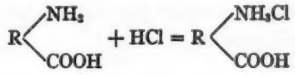
to the acid side by the sulfate and especially by the phosphate ions. This we ascribed to a decrease in the anionic dissociation of the sulfated and phosphated complexes.)

Apart from differences in the isoelectric points it was found that gelatin shows a distinctly different behavior from the other proteins. Thus the three proteins albumen, casein, and edestin become strongly negative and positive on the respective sides of the isoelectric point while gelatin remains electrokinetically very sluggish over the entire range. This is most clearly brought out by the flattened form of the gelatin curve in figure 11A. This would lead us to suspect that gelatin is less reactive both as an acid and as a base.

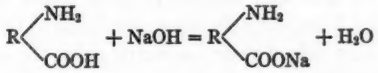
The curves in figure 11B express the buffer capacity of the four proteins. Albumen and casein show about the same capacity to neutralize acids whereas edestin is a decidedly stronger base which is in harmony with the higher isoelectric point of this protein. Gelatin, which was found isoelectric at a pH (5.2) as high as that of edestin shows the weakest buffer action for acids. This again, points to a less active protein. In our study of the proteinated complexes we shall find a consistent indication of this relative inactivity of gelatin. This property of gelatin might be related to its origin, namely, the connective tissues (collagen). Figure 11B does not bring out significant differences in the buffer action toward bases. To show this the pH must be brought to higher values, for although the proteins here studied are stronger as acids than as bases they are yet too weak as acids to neutralize much base except in alkaline solutions. The curves run accordingly more nearly parallel to the pH axis between the isoelectric point and a pH value about 9.0. The cation adsorption and exchange capacity by the proteins at neutrality must, therefore, be small as compared to that of the stronger soil acidoids.

PROTEINATED COMPLEXES

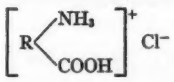
The now generally accepted view is that the proteins contain free amino and carboxyl groups reacting as bases:



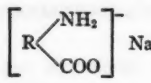
and as acids:



In the first case a positive protein ion or ion complex will be formed by dissociation thus:



and in the second case a negative protein ion or ion complex thus:



The isoelectric point must be that point at which an equal number of diffusible anions and cations are dissociated by the protein complex. It has been shown (12) that the soil complex contains diffusible anions and cations at the isoelectric point. This proves that the combination with acids and bases overlap, that is, some cations will displace H ions on the acid side and some anions will displace OH ions on the alkaline side of the isoelectric point. The same is probably true of the proteins, in which case the isoelectric condition represents the amphoteric ion $R\langle^{NH_3^+}_{COO^-}$. This might be looked upon as an inner salt which is completely ionized analogous to $CH_3\,COO^- + NH_4^+$ as pointed out by Michaelis (13b). But there can be only a small amount of these amphoteric ions because the proteins are very weak acids and bases and therefore extensively hydrolyzed into the molecular species $R\langle^{NH_2}_{COOH}$ analogous to the reaction: $NH_4^+ + CH_3\,COO^- \rightleftharpoons NH_3 + CH_3COOH$.

In acid solutions the ionization of the carboxyl group is suppressed while the ionization of the ammonium group is increased. The protein becomes then a positive ion or ion complex. In alkaline solutions or in solutions having a higher pH than that of the isoelectric point the ionization of the ammonium group is suppressed while the ionization of the carboxyl group is increased. The protein is therefore electronegative. As long as the compound formed with the added acid and base are highly ionized the isoelectric point will remain the same.

But if the acid or base (anion or cation) with which the protein combines forms a non-ionized compound then the aforementioned simple rule no longer applies. A new complex will be formed which possesses a different isoelectirc point. Some of these complexes which play an important rôle in the formation and properties of soils will now be discussed.

ALUMINUM AND IRON "PROTEINATES"

The sesquioxides of iron and aluminum were found to be isoelectric at pH values of 7.1 and 8.1 respectively when precipitated from the chlorides by NaOH (9). On the acid side of their isoelectric point the sesquioxides become increasingly electropositive as a result of the dissociation of the compound formed with the acid ion thus:

$$[(Al_2O_3)_{x-1}\cdot Al_2O_3]^{++} + 2Cl^-$$

and

$$[(Al_2O_3)_{x-2}\cdot 2Al_2O_3]^{++++} + 4Cl^- \text{ etc.}$$

The proteins which we have here studied were found to be isoelectric at pH values from 4.8 to 5.2. At higher pH values the proteins become increasingly negative as a result of the dissociation of the proteinate formed with the base (NaOH) thus:

$$[(NH_2)_n \cdot R \cdot (COOH)_{m-1}\ COO]^- + Na^+$$

and

$$[(NH_2)_n \cdot R \cdot (COOH)_{m-2}\ (COO)_2]^{--} + 2Na^+ \text{ etc.}$$

where R represents a protein molecule or molecular aggregate containing n free NH_2 groups and m free COOH groups.

Below a pH of 8.1 alumina exists as a cation while above a pH of 4.8 casein exists as an anion. Now since these ions of opposite charge are non-diffusible colloidal ions we should expect a double displacement to take place, within the pH range 4.8–8.1, upon mixing the two solutions resulting in the formation of aluminum "caseinate" (or more correctly, caseinated alumina) and NaCl. This combination would show itself in a complete or partial electrical neutralization of the colloids. A new complex would be formed having an isoelectric point somewhere between pH 4.8 and 8.1, the exact value depending entirely on the relative proportions of the two components.

Since alumina grows progressively more positive as we decrease the pH from 8.1 to 4.8 and since casein increases in negative strength in the opposite direction it is evident that the proportion of the two components which will yield an isoelectric complex at any particularlar pH will be inversely proportional to their "strength." Near pH 4.8 a little alumina and much casein will be required while near pH 8.1 it will require much alumina and little casein.

The important fact is that every proportion of alumina and casein can be isoelectrically precipitated. This must apply to all mixtures of amphoteric colloids although it has often been stated by several investigators, who have paid no attention to the pH, that such colloids are precipitated in certain proportions only.

In table 75 are shown the flocculation and cataphoresis at various pH values of three aluminum caseinate systems. Space does not allow a more extensive tabulation but the three systems are sufficient to show the law governing the reactions between amphoteric colloids having different isoelectric points.

The results are graphically shown in figure 12. The relationship between composition and the isoelectric pH is more strikingly brought out by including in the figure the curves for "free" alumina on the one side and "free" casein on the other.

It will be noted that a considerable quantity of protein is required to lower the isoelectric pH of alumina while, conversely, small amounts of alumina cause a considerable increase in the isoelectric pH of the protein. This is, of course, in the first place, due to the high equivalent weight of the protein and, in the second place, to the weakness of the protein as an acid so near its iso-

electric point. With 5 millimols $AlCl_3$ to 1 gm. casein the isoelectric point is at pH 7.4 and with as little as 1/3 millimol $AlCl_3$ (about 0.017 gm. Al_2O_3 to a gm. of casein) the complex is isoelectric at pH 5.5. The actual composition of the isoelectric complexes is given in table 81a, and the relation between the

TABLE 75

The $AlCl_3$ + Na −Caseinate systems

SOLUTION A, 20 cc. + HCl*	SOLUTION B, 20 cc. + NaOH*	FLOCCULATION		μ/SEC. 1 VOLT/CM.	pH
		After mixing	Overnight		
millimol	*millimol*				
		No. 121			
A: 5 millimols $AlCl_3$ in 1,000 cc. B: 1 gm. casein + 1 millimol NaOH in 1,000 cc.					
0.0	0.25	Opal	0	.....	6.2
0.0	0.26	Instant	++++	+1.21	6.6
0.0	0.27	Instant	++++	±0.0	7.4
0.0	0.275	Instant	++++	−0.34	7.6
0.0	0.28	Instant	++++	−0.92	7.9
0.0	0.30	Opal	0	.....	8.8
		No. 121			
A: 1 millimol $AlCl_3$ in 1,000 cc. B: 1 gm. casein + 1 millimol NaOH in 1,000 cc.					
0.0	0.0325	Opal	++	+1.35	6.0
0.0	0.035	Opal	++++	+0.55	6.3
0.0	0.0375	Rapid	++++	+0.17	6.55
0.0	0.040	Instant	++++	−0.40	6.7
0.0	0.0425	Slow	++	−0.98	6.8
0.0	0.050	Opal	0	.....	7.15
		No. 123			
A: ⅓ millimol $AlCl_3$ in 1,000 cc. B: 1 gm. casein + 1 millimol NaOH in 1,000 cc.					
0.015	0.0	Opal	0	.....	4.8
0.010	0.0	Slow	++++	+0.61	5.2
0.0075	0.0	Instant	++++	±0.0	5.5
0.005	0.0	Instant	++++	−0.67	5.75
0.0025	0.0	Rapid	+++	−0.87	5.9
0.0	0.0	Turbid	++	−1.08	6.2
0.0	0.005	Opal	0	.....	6.6

* Plus enough water to make 50 cc.

isoelectric composition and the pH is shown graphically in figure 14, both of which will be discussed later.

Compared to the humate complex the proteins are therefore much weaker as acids and as electronegative colloids. One millimol of $AlCl_3$ to 1.05 gm.

humus resulted in a complex which was electronegative at all pH values, whereas 2 millimols $AlCl_3$ to the same amount of humus yielded an isoelectric complex at pH about 4.5 (10).

Experiments with $FeCl_3$ instead of $AlCl_3$ have shown that, as in the case of the silicated, phosphated, and humated complexes, the proteinated ferric complexes are isoelectric at a lower pH than the corresponding Al-complexes.

Albumin, edestin, and gelatin were studied in a single proportion with $AlCl_3$, namely in the ratio of 1 millimol $AlCl_3$ to 1gm. protein. The results are shown in table 76.

It will be noted that both edestin and gelatin, which in the "free" condition are isoelectric at a higher pH than either casein or albumin, and are therefore more weakly acidic, suppress the isoelectric pH of their aluminum complexes less than the latter proteins. Such differences in the relative strength of the

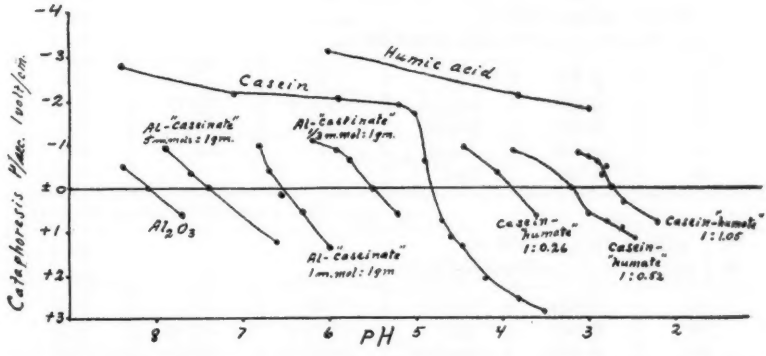


FIG. 12. CATAPHORESIS AND ISOELECTRIC POINTS OF A SERIES OF Al-"CASEINATES" AND OF CASEIN-"HUMATES"

acid and basic groups of the proteins express themselves consistently, especially in the case of edestin and casein, which have been subjected to a comparative study (see figure 13). Gelatin has been found to behave somewhat erratically, probably because of hydrolysis which this protein rapidly undergoes in spite of the fact that the solutions were kept only a short time and that microbial activity was inhibited by the addition of toluene.

The property of gelatin of not coagulating at its isoelectric point reflects itself in the Al-"gelatinate." A mere opalescence but no flocculation was observed in any of the tubes after mixing. But on the following day flocculation was complete near the isoelectric point. The figures in table 81A show, however, that only about 25 per cent of the gelatin was carried down as compared to about 90 per cent of the other proteins. This lack of reactivity of the gelatin, which has already been referred to, expresses itself in all the complexes studied.

The question as to the nature of the compounds formed in the preceding

reactions will be discussed later. It is desirable first to discuss another type of proteinated complexes.

THE PROTEIN "HUMATES"

In the preceding chapter we have studied the behavior of proteins as negative colloids, as acids, and as anions. We shall now bring the same proteins

TABLE 76

The $AlCl_3$ + Na-albuminate, Na-edestinate, and Na-gelatinate systems

SOLUTION A	SOLUTION B, 20 CC. + NaOH	FLOCCULATION		μ/SEC. 1 VOLT/CM.	pH
		After mixing	Overnight		
cc.	*millimol*				
		No. 125			
A: 1 millimol $AlCl_3$ in 1,000 cc. B: 1 gm. albumen + 1 millimol NaOH in 1,000 cc.					
20	0.030	Opal	0	.....	5.8
20	0.035	Instant	++++	+0.15	6.1
20	0.0375	Instant	++++	−0.42	6.4
20	0.040	Rapid	+++	−0.72	6.6
20	0.0425	Opal	0	.....	6.8
		No. 124			
A: 1 millimol $AlCl_3$ in 1,000 cc. B: 1 gm. edestin + 1 millimol NaOH in 1,000 cc.					
20	0.040	Opal	0	.....	6.3
20	0.045	Slow	++++	+0.92	6.65
20	0.0475	Instant	++++	+0.47	7.0
20	0.050	Instant	++++	±0.0	7.15
20	0.0525	Slow	++++	−0.45	7.4
20	0.060	Opal	+	−1.01	7.7
		No. 126			
A: 1 millimol $AlCl_3$ in 1,000 cc. B: 1 gm. gelatin + 1 millimol NaOH in 1,000 cc.					
20	0.050	Clear	0	.....	6.9
20	0.0525	Opal	++	+0.40	7.0
20	0.055	Opal	++++	+0.18	7.15
20	0.0575	Opal	++++	− slight	7.6
20	0.060	Opal	+++	−0.26	7.8
20	0.065	Clear	++	−0.76	8.2

below their isoelectric pH and study them as positive colloids, as bases, and as cations. In this condition we shall find them to combine with negative colloids, with acids, and with anions. If the substance we introduce into a solution of a positive protein yields anions or anion complexes, which form

with the protein a non-ionized complex, then we shall find that the isoelectric point of this complex is lower on the pH scale than that of the original protein.

TABLE 77

The casein-"humate" systems

SOLUTION A, 20 cc. + HCl	SOLUTION B	FLOCCULATION		μ/SEC. 1 VOLT/CM.	pH
		After mixing	Overnight		
millimol	*cc.*				
		No. 131			
A: 0.5 gm. casein + 0.5 millimol NaOH in 1,000 cc. B: 1.5 m. equiv. Na-humate in 1,000 cc.					
0.04	20	Opal	0	.....	....
0.06	20	Slow	++++	−0.82	3.1
0.08	20	Instant	++++	−0.61	2.9
0.10	20	Instant	++++	−0.32	2.85
0.13	20	Instant	++++	±0.0	2.75
0.18	20	Instant	++++	+0.30	2.6
0.28	20	Instant	++++	+0.76	2.2
		No. 132			
A: 1 gm. casein + 1 millimol NaOH in 1,000 cc. B: 1.5 m. equiv. Na-humate in 1,000 cc.					
0.04	20	Opal	0	−1.78	....
0.06	20	Rapid	++++	−0.87	3.9
0.08	20	Instant	++++	±0.0	3.2
0.10	20	Instant	++++	+0.61	3.0
0.14	20	Instant	++++	+0.76	2.8
0.18	20	Slow	++++	+0.90	2.6
0.28	20	Opal	++	+1.16	....
		No. 133			
A: 1 gm. casein + 1 millimol NaOH in 1,000 cc. B: 0.75 m. equiv. Na-humate in 1,000 cc.					
0.04	20	Opal	0	.....	....
0.045	20	Rapid	++++	−0.93	4.45
0.05	20	Instant	++++	−0.34	4.05
0.06	20	Instant	++++	+0.64	3.6
0.08	20	Slow	++	+1.11	....
0.10	20	Turbid	+	+1.17	....

For the study of such complexes in which the protein plays the part of the basic group we have selected the following soil acidoids: humic acid from peat, bentonite, and silicic acid.

The humic acid was employed in the form of Na-"humate" containing 0.35 gram dry weight humus to each millimol NaOH. The same preparation has

been described in section IV of this series. The flocculation and cataphoresis at various pH values of three different mixtures of casein and Na-humate are

TABLE 78

The albumin-edestin- and gelatin-"humate" systems

SOLUTION A, 20 cc. + HCl	SOLUTION B	FLOCCULATION		μ/SEC. 1 VOLT/CM.	pH
		After mixing	Overnight		
millimol	*cc.*				
		No. 135			
A: 1 gm. albumin + 1 millimol NaOH in 1,000 cc. B: 1.5 m. equiv. Na-humate in 1,000 cc.					
0.06	20	Opal	0	−1.44	....
0.065	20	Rapid	++++	−0.87	4.0
0.07	20	Instant	++++	−0.52	3.8
0.08	20	Instant	++++	±0.0	3.4
0.09	20	Instant	++++	+0.52	3.25
0.10	20	Slow	++++	+0.70	3.15
0.12	20	Turbid	+++	+0.95	3.0
		No. 134			
A: 1 gm. edestin + 1 millimol NaOH in 1,000 cc. B: 1.5 m. equiv. Na-humate in 1,000 cc.					
0.05	20	Opal	+	−1.51	....
0.055	20	Instant	++++	−1.26	4.55
0.06	20	Instant	++++	−0.47	4.1
0.07	20	Instant	++++	+0.46	3.5
0.08	20	Rapid	++++	+0.78	3.35
0.09	20	Rapid	++++	+1.01	3.25
0.10	20	Turbid	+++	+1.21	3.15
		No. 136			
A: 1 gm. gelatin (no NaOH) in 1,000 cc. B: 1.5 m. equiv. Na-humate in 1,000 cc.					
0.03	20	Opal	0	.....	....
0.035	20	Instant	++++	−slight	4.2
0.04	20	Instant	++++	+slight	3.9
0.045	20	Instant	++++	+0.43	3.7
0.05	20	Slow	+++	+0.50	3.5
0.06	20	Opal	+	+0.90	....

shown in table 77. Table 78 shows the same relationship with albumin, edestin, and gelatin in a single proportion of each with the humate.

In the aluminum "proteinate" systems we were dealing with reactions between two amphoteric components. The isoelectric point of any complex of these two components will be found between the isoelectric points of the pro-

tein and of the alumina. In the protein-"humate" systems we are dealing with the reactions between an amphoteric component on the one side and a strictly electronegative and acidic component on the other. The result of this reaction will be as follows: As the humus or the negative component is increased the isoelectric point will be displaced more and more to the acid side until, with a certain excess of the humus or negative component, the resulting complex is no longer electrokinetically amphoteric but migrates anodically at all pH values.

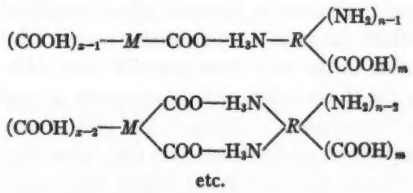
The protein-"humate" systems resemble in behavior the aluminum and ferric "humates" which were discussed in previous sections (10), but the isoelectric points of the former are, of course, much lower because of the weaker basic properties of the proteins. The relationship is best seen in figures 8 and 12. In figure 12 the cataphoresis of humic acid in HCl solution is also shown. In system no. 131, in which the proportion of humate to casein is the highest, we note that the positive charge is low and that the complex is completely flocculated at a pH as low as 2.2. When the proportion of humus exceeds a certain limit the complex must remain negative and approach in behavior that of "free" humus.

If we assume the "humic acid" to be strictly acidic [which is not true because all humus contains some nitrogen, indicating proteins, and although the peat humus here employed is cataphoretically negative at all pH values its amphoteric reaction was demonstrated by an exchange alkalinity (12) at low pH values], we might represent it by a general formula if we make the further assumption that its acidic property is due to COOH groups thus: $M(COOH)_x$ and its sodium compounds thus:

$$[M\cdot(COOH)_{x-1}\cdot COO]^- + Na^+$$

$$[M\cdot(COOH)_{x-2}\cdot 2COO]^{--} + 2Na^+$$

etc., in which M represent the molecule or molecular aggregate to which x groups of COOH are linked. The complexes which the positive proteins form with humic acids may then be expressed by the formulas:



etc.

These complexes would react amphoterically and become positively charged in solutions sufficiently acid to yield a greater number of the cationic species, $[R-NH_3]^+$ than of the anionic species, $[R-COO]^-$.

The pH at which the two forms of dissociation balance each other must

be the isoelectric pH. Above this pH the complex would be negative because of preponderance of the anionic species. A complex in which the non-diffusible acidic component (e.g. the humus) exceeds the non-diffusible basic component (e.g. the protein) must remain negative at all pH values either because all the basic groups are linked up in a non-ionizing combination thus:

$$(COOH)_{z-n}—M—(COO)_n—(H_2N)_n—R—(COOH)_m$$

or because the number of the NH_2 groups, free to combine with the diffusible acid (e.g. HCl), is insufficient to form the required excess of the cationic species. In other words the anionic dissociation by the complex would never exceed the cationic dissociation at any pH.

It is interesting to note that gelatin, which, when alone, does not flocculate even at its isoelectric point, is instantly flocculated together with humic acid. The amount of gelatin carried down in the floc is, however, in every case less than that of any other of the proteins (see table 81b). When we speak about gelatin or any other protein as a "protective" colloid we should be careful to define the conditions and keep in mind the chemical nature of the substance to be "protected."

THE PROTEIN "BENTONATES"

It is realized that the term "bentonate" is a very loose one but we shall find it expedient to use it just as, and for the same reason that, we have found it expedient to employ the term "humate." Bentonite is distinctly acidic and gives no indications of being amphoteric either cataphoretically or in its exchange reactions (12). Electrodialyzed, its suspension was found to be more acid (pH 2.6) than that of the aforementioned sample of humus but the cation exchange capacity of the bentonite at pH 7.0 is only a little more than one-third of that of the humus, or 1.02 and 2.87 milliequivalents per gram respectively. At a pH of 2.0 the bentonite adsorbed, however, considerable Ca ions (12) whereas the Ca-ion adsorption by humus reached a vanishing point at this pH. Bentonite must therefore be looked upon as a stronger acid than humus. This can partly be ascribed to the presence of proteins in the latter. The equivalent weight of the humus is, however, about one-third that of bentonite and possesses therefore the greatest neutralizing power. All this is mentioned because of its relation to the way these acidoids react with the proteins.

Table 79 shows the flocculation and cataphoresis at various pH values of casein and edestin "bentonates." Albumin and gelatin were also included but except for the composition of the isoelectric floc (table 81c) the tabulation is omitted. The bentonite was used in the highly dispersed Na-saturated condition.

All the proteins flocculate isoelectrically with bentonite at a pH below that of the "free" proteins. But the displacement of the isoelectric point is not nearly as great as in the case of the "humates." With a ratio of 1:1 of casein to bentonite the isoelectric point is displaced from about 4.8 to about 4.25

(interpolated). With the same ratio of edestin to bentonite the isoelectric point was displaced from 5.2 to about 4.6. With a ratio of 2 of protein to 1.05 of humus the isoelectric points were displaced to pH 3.2 in the casein and 3.8 in the edestin system. With a ratio of 1 casein to 1.05 humus the system was isoelectric as low as pH 2.75.

The humus with nearly three times the neutralizing power per gram as compared to bentonite, causes by far the greatest displacement of the iso-

TABLE 79

The casein- and edestin-"bentonate" systems

SOLUTION A, 20 cc. + HCl	SOLUTION B	FLOCCULATION		μ/SEC. 1 VOLT/CM.	pH
		After mixing	Overnight		
millimol	*cc.*				
		No. 111			
A: 1 gm. casein + 1 millimol NaOH in 1,000 cc. B: 1 gm. bentonite in 1,000 cc.					
0.035	20	Opal	0	+1.89	....
0.040	20	Instant	+++	−1.68	5.6
0.045	20	Instant	++++	−1.38	5.5
0.050	20	Instant	++++	−0.25	4.4
0.060	20	Instant	++++	+0.70	3.9
0.080	20	Rapid	++	+1.48	....
0.120	20	Slow	+	+1.65	....
		No. 112			
A: 1 gm. edestin + 1 millimol NaOH in 1,000 cc. B: 1 gm. bentonite in 1,000 cc.					
0.025	20	Turbid	0	−1.78 (?)	....
0.030	20	Instant	++	−1.89 (?)	5.9
0.035	20	Instant	+++	−1.55	5.7
0.040	20	Instant	++++	−1.10	5.3
0.045	20	Instant	++++	−0.30	4.65
0.050	20	Rapid	+++	+1.12	4.4
0.060	20	Slow	++	+1.89	4.35
0.120	20	Turbid	0	+1.89	....

electric point of the proteins to the acid side. This speaks strongly in favor of an actual combination between the basic group in the proteins and the acid group of the humus and bentonite as suggested in the foregoing. A more direct proof of such combination will be given later.

THE PROTEIN SILICATES

Silicic acid is an extremely weak acid and adsorbs only small amounts of cations from neutral salt solutions. Its cation exchange capacity is therefore

very small as compared to the hydrous alumino-silicates such as bentonite and the synthetic silicates discussed in the foregoing sections (11). Why silicic acid should be a stronger acid in (partial) combination with Al and Fe than in the free state has never been explained. The structure might be a factor, as previously suggested (11), but it might be that silicic acid is a pseudo-acid existing in water largely as SiO_2 and only to a small extent as H_2SiO_3 or H_4SiO_4. In partial combination with the hydroxides of Al and Fe thus:

$$(OH)_2—Al—SiO_3—H$$

TABLE 80

The casein and edestin "silicate" systems

SOLUTION A, 20 cc. + HCl	SOLUTION B	FLOCCULATION		μ/SEC. 1 VOLT/CM.	pH
		After mixing	Overnight		
millimol	*cc.*				
		No. 115			
A: 1 gm. casein + 1 millimol NaOH in 1,000 cc. B: 10 millimols Na_2SiO_3 in 1,000 cc.					
0.460	20	Opal	0	.....	5.35
0.465	20	Slow	++++	−0.73	4.9
0.467	20	Rapid	++++	±0.0	4.7
0.470	20	Rapid	++++	+0.47	4.5
0.475	20	Opal	+	.....	4.35
0.48	20	Opal	0	.....	4.25
		No. 116			
A: 1 gm. edestin + millimol NaOH in 1,000 cc. B: 10 millimols Na_2SiO_3 in 1,000 cc.					
0.440	20	Opal	0	.....	6.3
0.450	20	Milky	+	.....	5.85
0.455	20	Instant	++++	−1.50	5.65
0.4575	20	Instant	++++	−0.76	5.4
0.460	20	Instant	++++	−0.43	5.25
0.465	20	Milky	+++	+1.01	5.0
0.47	20	Opal	0	.....	4.8

a reversion to the SiO_2 could not take place thus leaving a stabilized acid residue. This problem is now being investigated in the form of a series of alumino-silicates of great variations in composition.

The weakness of silicic acid expresses itself interestingly in its reaction with the proteins. The results with casein and edestin are shown in table 80. Albumin reacted in a similar manner but gelatin, which flocculated more or less hesitatingly in the preceding systems, was entirely unaffected by silicic acid as far as the optical appearance went.

The weak silicic acid which adsorbs only small amounts of the common cations from neutral solutions combines evidently very feebly with the protein cations at the low pH at which these substances become cationic. The isoelectric point of the proteins is displaced to the acid side in a barely perceptible degree by as much as 10 millimols of silicic acid per gram protein.

The displacement is best seen in figure 13, in which the various casein and edestin systems are compared. The comparison is instructive because it shows fairly constant differences in the isoelectric points of the two series of proteinated

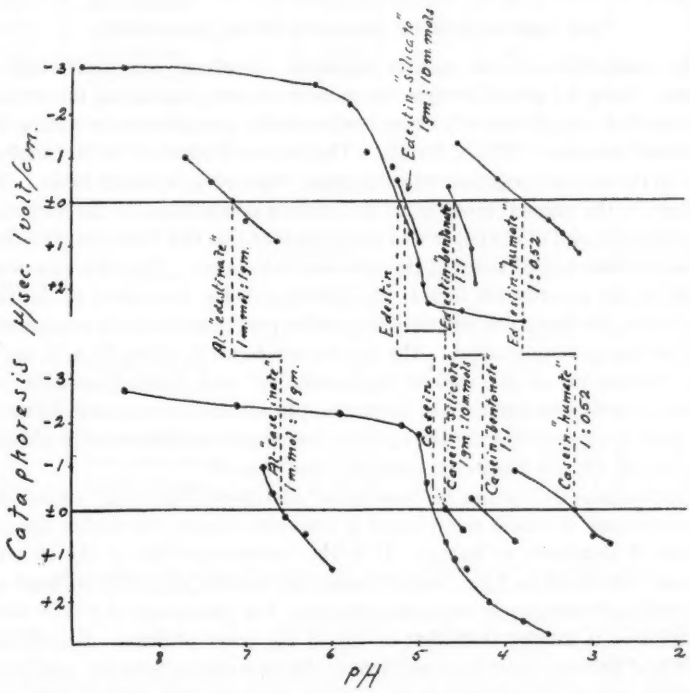


FIG. 13. THE ISOELECTRIC POINTS OF CASEIN AND EDESTIN AND SOME OF THEIR COMPOUNDS, COMPARED

complexes. The broken line perpendiculars indicate the differences of the two systems. The isoelectric points of "free" edestin and casein differ by about 0.4 pH. This order of magnitude is, within certain limits, maintained throughout the two series.

This all speaks well in favor of the accuracy of the method here employed in measuring the cataphoretic movement of ultramicroscopic particles. The cell constructed by the author has the great advantage of enabling the experimenter to make a great number of measurements in a short time. Mass work

of this kind is, however, unavoidably done at the expense of great accuracy. Greater accuracy may be attained but only at the expense of much time. The sources of errors in the foregoing measurements are not entirely cataphoretic but of the most intricate and varied origin. Thus besides errors in pH the systems are affected by the mode of mixing the colloids, but the drift in the magnitude of the charge and of the isoelectric point, by hydrolysis, by the temperature, etc. The systems are dynamic and unstable, the colloidal complex, especially the amphoteric complex, is very sensitive.

THE COMPOSITION OF THE ISOELECTRIC COMPLEXES

The composition of the various isoelectric complexes was determined as follows: Using 0.2 gm. of protein, the various systems, containing the proportions studied cataphoretically, were isoelectrically precipitated by adding the calculated amount of HCl or NaOH. The floc was filtered off but not washed, dried in the air, and, together with the paper, digested in Kjeldahl flasks. The nitrogen in the original proteins, in the various precipitates, in the humus, in the bentonite, and in the paper was determined and on this basis the quantities of the proteins carried down in the floc were calculated. The SiO_2 was determined in the supernatant liquid, the difference being accounted to the floc. The humus, the bentonite, and the sesquioxides were quantitatively precipitated in all of the systems studied. The results are shown in tables 81 a, b, and c. The composition of the various Al-"caseinates" and casein-"humates" are plotted as ordinates against the isoelectric pH values as abscissas in figure 14. The curves, representing the two series of compounds, originate on the abscissa axis, at pH 4.8, the isoelectric point of "free" casein.

If we examine the various Al-"caseinates" and casein "humates" we find that the percentage of casein precipitated is somewhat higher, the higher the proportion of aluminum or humus. It is also noteworthy that of the 10 millimols of SiO_2 added to 1 gm. each of casein and edestin only 0.84 millimol and 0.63 millimol respectively were precipitated. The percentage of gelatin which precipitated is smaller than that of any of the other proteins. Gelatin and silicic acid did not, under the conditions of the experiment, form any precipitate although such a percipitation is recorded by Graham, who worked with more concentrated solutions (1).

We note that in no case is the protein quantitatively precipitated. Whether the precipitated and non-precipitated fractions of the proteins are identical or of a different nature has not been determined. It is conceivable that the fraction which remains in solution represents, on the one side a basic group displaced by Al and Fe and on the other side an acid group displaced by the humus and bentonite.

The so-called "simple" proteins may constitute compounds of proteins having different isoelectric points just as the "conjugated" proteins are compounds of proteins with non-protein groups. Thus the basic histones would undoubtedly combine with the acidic prolamines forming complexes which would all be

TABLE 81A

The composition and pH of isoelectric Al- and Fe-"proteinates"

(Dilution = 1 gm. protein in 2,500 cc.)

SYSTEM NUMBER	COMPLEX	COMPOSITION RATIO OF SYSTEM		ISOELECTRIC pH	PROTEIN PRECIPITATED	COMPOSITION OF ISOELECTRIC COMPLEX	
		R_2O_3	Protein			R_2O_3	Protein
		millimol	*gm.*		*per cent*	*millimol*	*gm.*
127	Fe-caseinate	1	0.40	7.0	92.8	1	0.37
121	Al-caseinate	1	0.40	7.4	89.2	1	0.36
123	Al-caseinate	1	6.00	5.5	81.5	1	4.89
122	Al-caseinate	1	2.00	6.6	84.2	1	1.68
124	Al-edestinate	1	2.00	7.15	90.5	1	1.81
125	Al-albuminate	1	2.00	6.2	92.5	1	1.85
126	Al-gelatinate	1	2.00	7.4	25.4	1	0.51

TABLE 81B

The composition and pH of isoelectric protein "humates"

SYSTEM NUMBER	COMPLEX	COMPOSITION RATIO OF SYSTEM		ISO-ELECTRIC pH	PROTEIN PRECIPITATED	COMPOSITION OF ISOELECTRIC COMPLEX	
		Protein	Humus			Protein	Humus
		gm.	*gm.*		*per cent*	*gm.*	*gm.*
131	Casein-humate	1.00	1.05	2.75	95.0	0.905	1.00
133	Casein-humate	1.00	0.262	3.9	89.9	3.43	1.00
132	Casein-humate	1.00	0.525	3.2	93.6	1.78	1.00
134	Edestin-humate	1.00	0.525	3.8	94.7	1.80	1.00
135	Albumin-humate	1.00	0.525	3.4	94.7	1.80	1.00
136	Gelatin-humate	1.00	0.525	4.0	70.2	1.34	1.00

TABLE 81C

The composition and pH of isoelectric protein-"bentonates" and "silicates"

SYSTEM NUMBER	COMPLEX	COMPOSITION RATIO OF SYSTEM		ISO-ELECTRIC pH	PROTEIN PRECIPITATED	COMPOSITION OF ISOELECTRIC COMPLEX	
		Protein	Bentonite			Protein	Bentonite
		gm.	*gm.*		*per cent*	*gm.*	*gm.*
111	Casein-bentonate	1.00	1.00	4.25	95.1	0.951	1.00
112	Edestin-bentonate	1.00	1.00	4.58	94.5	0.945	1.00
113	Albumin-bentonate	1.00	1.00	4.20	97.7	0.977	1.00
			SiO_2			SiO_2 PRECIPITATED	
			millimols			*per cent*	
115	Casein-silicate	1.00	10.00	4.7	79.5	8.4	
116	Edestin-silicate	1.00	10.00	5.15	82.0	6.3	

isoelectric at pH values between those of the "simple" proteins. Such combination must result within the animal and plant organism. For this reason

no two samples of any one kind of protein can be expected to be exactly alike. It might be said about the proteins that they are products of their environment.

The chemistry of the proteins which, at the time of Emil Fischer, looked so promising has made very little progress and, in the opinion of as eminent an authority as Levene (3), promises little for the future. But where the methods which have proved so useful in the organic chemistry of the more simple compounds fail, the methods of colloid chemistry might be successfully applied.

THE NATURE OF THE COMPOUNDS

The most important question arising from the preceding data is the mechanism by which the various components react to form new complexes. The problem of the mutural flocculation of oppositely charged colloids has been

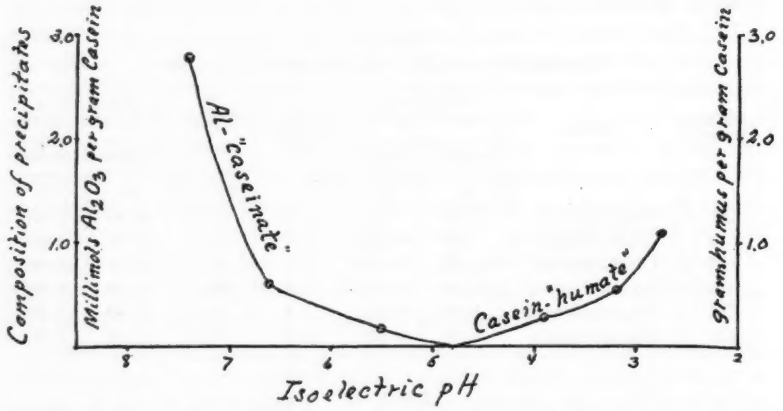


FIG. 14. RELATION BETWEEN COMPOSITION AND ISOELECTRIC pH OF Al-"CASEINATE" AND CASEIN-"HUMATE"

extensively investigated and many of the investigators are satisfied with the explanation that the colloid, being stabilized by its charge, must flocculate when this charge is neutralized. Some others (15) would explain the phenomenom by assuming that the charge and stability of the colloid are due to an extraneous stabilizing electrolyte and that the electrical neutralization and flocculation is the result of a chemical reaction between the stabilizing electrolytes. By what valences or forces the stabilizing electrolyte is attracted to the colloid in the first place and what satisfies these valences after the stabilizer is removed are not considered. The colloids themselves do not react, they are not ionogens but merely carriers of ions.

Although this explanation may be true of inert substances such as paraffin, collodion, and air bubbles, it does not fit the case of reactive colloids like the acidoids, ampholytoids, and their compounds. We might as well say that

NaOH stabilizes CO_2, that HCl stabilizes CaO, and that, when the solutions are mixed, a precipitate is formed because the stabilizers react, as to say that NaOH stabilizes a protein, that HCl (or $AlCl_3$) stabilizes alumina, and that, when the two are mixed, flocculation occurs because a chemical reaction removes the stabilizers. We would be stating the most unessential half of the truth, for we would have said nothing about calcium carbonate and nothing about the complex of alumina and protein which was formed by the double displacement.

If alumina, or rather aluminum oxychloride, is positive thus:

$$[(Al_2O_3)_x—Al_2O_3]^{++}\ 2Cl^-$$

TABLE 82

The adsorption of SO_4 ions by alumina and aluminum-"albuminate" at various pH

A's: 0.5 millimol $Al_2(SO_4)_3$ in 250 cc.

B's: 0.5 millimol $Al_2(SO_4)_3$ + 1 gm. albumin in 250 cc.

SOLUTION	NaOH	pH	SO_4		Al_2O_3	
			In solution	In precipitate	In solution	In precipitate
	millimols		*millimols*	*millimol*	*millimol*	*millimols*
A1	1.75	4.25	1.26	0.24	0.122	0.378
A2	2.00	4.4	1.24	0.26	0.075	0.425
A3	2.50	6.45	1.31	0.19	None	0.500
B1	1.75	4.7	1.27	0.23	0.059	0.441
B2	2.00	5.0	1.285	0.215	0.018	0.482
B3	2.25	5.9	1.35	0.15	None	0.500

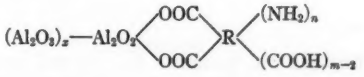
Composition of precipitates:

A1: $Al_2O_3 \cdot (SO_3)_{0.635}$ B1: $Al_2O_3 \cdot (SO_3)_{0.516}$

A2: $Al_2O_3 \cdot (SO_3)_{0.618}$ B2: $Al_2O_3 \cdot (SO_3)_{0.447}$

A3: $Al_2O_3 \cdot (SO_3)_{0.375}$ B3: $Al_2O_3 \cdot (SO_3)_{0.305}$

and is rendered isoelectric by the removal of the dissociated Cl ions then some other anions which do not dissociate must take their place. In the case of the Al-"proteinates" it is assumed that the protein anions have displaced the diffusible Cl ions thus:



Now this complex can combine with more Cl at a lower pH and again become positive, but at a given pH the proteinated complex should contain less Cl than the free alumina, provided the pH is not so low that the amino groups combine with appreciable quantities of HCl. To verify this the pH must therefore be kept well above the isoelectric point of the protein.

This has been investigated, but instead of the Cl ion the SO_4 ion was selected

because the latter ion is more strongly adsorbed by alumina and, what is more important, this divalent anion keeps the positive complex flocculated over a much greater range. Aluminum sulfate was therefore used instead of the chloride. Albumin was used because this protein was free from both calcium and sulfates. The results are shown in table 82.

The experiment shows clearly that the protein, reacting as anions, displaces the SO_4 ions just as we found the silicate ions to do in the silicated complexes (9). Since the pH values of the A and B solutions are not the same, the

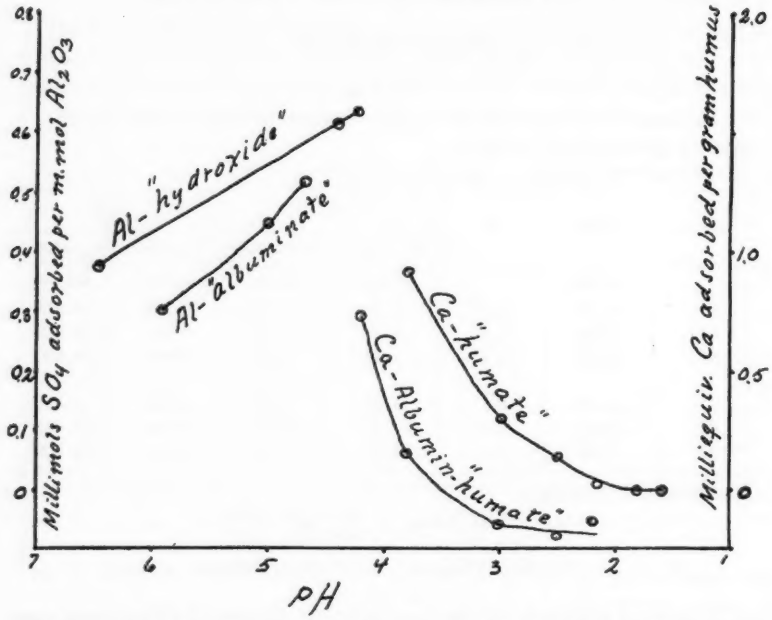


FIG. 15. ALUMINUM "HYDROXIDE" COMBINES WITH (ADSORBS) MORE SO_4 THAN Al-ALBUMINATE (LEFT); HUMIC ACID ADSORBS MORE CA THAN ALBUMIN "HUMATE" (RIGHT)

This proves that protein anions and cations displace the common anions and cations respectively.

relationship is best brought out in figure 15. It will be noted that the "albuminate" curve is such that if extended on the acid side it would cross the free alumina curve. This is what we should expect, because at lower pH values the amino group becomes active and adsorbs anions.

We shall now see that the protein cations displace the common cations in combination with acidoids. If the proteins react as cations on the acid side of their isoelectric point and combine as such with the acidoid group, i.e., humus, bentonite, etc. then at a given (low) pH the acidoids should adsorb

more of the common cations when alone than when in the presence of proteins, that is, Ca-"humate" should contain more Ca at a pH of 3 or 4 than "Ca-albumin-humate."

To verify this the experiment shown in table 83 was performed. Allowance for the volume occupied by the solids was made by assuming a specific gravity of 1.4 for the organic matter.

At the same pH considerably more Ca was adsorbed by the free humus than by the mixture of albumin and humus. The results are best shown in figure 15. Here again the two curves show a tendency to cross and undoubtedly would cross at higher pH values where the protein is anodic and combines with cations. Since the systems do not flocculate except within the range covered, this could not be verified.

The negative adsorption in two of the albumin-"humate" systems is due to the Donnan distribution of the free ions. The swarm of Cl ions surrounding

TABLE 83

The adsorption of Ca ions by humus and by albumin-"humate" at various pH

A's: 3 m. equiv. Na-humate (= 1.05 gm. humus) + 4 m. equiv. $CaCl_2$ in 500 cc.
B's: 3 m. equiv. Na-humate + 2 gm. albumin + 4 m. equiv. $CaCl_2$ in 500 cc.

SOLUTION	HCl	pH	Ca IN SOLUTION	Ca ABSORBED
	m. equiv		*m. equiv.*	*m. equiv.*
A1	2.0	3.8	3.07	0.93
A2	4.0	3.0	3.70	0.30
A3	6.0	2.5	3.86	0.14
A4	8.0	2.15	3.97	0.03
A5	12.0	1.8	4.00	0.00
B1	3.0	4.2	3.27	0.73
B2	4.0	3.8	3.85	0.15
B3	6.0	3.0	4.14	−0.14
B4	10.0	2.5	4.19	−0.19

the positive complexes forces an excess of the free $CaCl_2$ into the external solution. This negative adsorption must be present in all of the other systems as well, except (theoretically) in the isoelectric, but is here more than balanced by the positive adsorption (7).

STOICHIOMETRY

The ion-complexes or micelles of the preceding compounds are highly multivalent, running perhaps in the larger ultramicrons, into the hundreds of thousands (8). The number of combinations is infinite. As previously pointed out, there can therefore be no stoichiometrical relationship between the constituent parts of so complex substances. But is not such a relationship an exception rather than a rule even in the case of "simple" compounds? Within the crystal lattice the law of definite proportions of any pair of ions doubtless

holds, but in the solution and at the surface of crystals or at the interface of colloids the composition of compounds may be as complex as the solution itself. We know that ions combine in some way with the water molecules (hydration) but we do not know in what proportions. We know that the ions in solution mutually affect one another but we know very little about the form in which they exist. Modern chemists frequently disagree as to the definition of acids, bases, and salts (16).

The mass law has been found to be only an approximation. The pH of a solution does not express the mass or concentration of the H ions but instead the activity of these ions. The energy or activity concept has taken the place of the mass concept in chemical reactions, and the law of definite and multiple proportions, being essentially a mass law, must likewise be modified by the newer concepts of energetics.

But, it might be argued, the stoichiometric relationship of "pure" compounds is the basis of all methods of analysis. The answer to this is, in the first place, that in the development of analytical methods every conceivable procedure is tried and only that one which yields the comparatively best results is adopted. The result is, in general, a method which is limited to a very narrow set of conditions. We are cautioned against the "occlusion" of this or that ion. In the second place the accuracy of the final determination of the separated "pure" compound is subject to inherent limitations. Thus the SO_4 or Ba ions are measured in terms of barium sulfate, which is always expressed by the formula $BaSO_4$. In other words the barium sulfate precipitate, whatever it is, is used as its own yardstick; we are determining the accuracy of the yardstick by itself.

THE REACTION WITH SILVER CHLORIDE

Silver chloride must be looked upon as one of the most simple compounds. It plays an important rôle in qualitative as well as in quantitative chemistry and respresents, to the analytical chemist, something very definite. Yet the complexity of this substance is easily demonstrated. It probably plays a minor rôle among the soil colloids but from what we have learned during the last years about the rarer elements any positive statement would be premature. But because of its bearing on the subject here studied a brief discussion will be given.

If an excess of NaCl is added to a dilute solution of $AgNO_3$ an opalescent, negative sol is formed. With an excess of $AgNO_3$ an opalescent, positive sol results. In equivalent proportions an isoelectric or nearly isoelectric precipitate is formed. This behavior, characteristic of the silver halides in general, has been extensively investigated by Lottermoser (4).

If in a series of beakers we add an increasing excess of NaCl in one direction and an increasing excess of $AgNO_3$ in the other and examine the sols thus formed we find that the dispersibility increases, as shown by a decreasing opalescence, as the excess of either reagent is increased. If we examine the various sols cataphoretically we find that the negative charge increases progessively

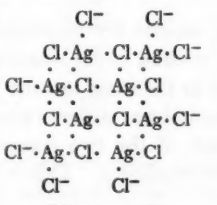
with an increase of NaCl whereas the positive charge increases with an increase of $AgNO_3$ (see table 84).

If we study the effect of these sols on a protein solution such as that of gelatin we find that the negative silver chloride sols precipitate with positive gelatin, i.e., on the acid side of the isoelectric point of the "free" protein, whereas the positive silver chloride sols precipitate with the gelatin on the alkaline side of its isoelectric point, i.e., with negative gelatin. Since the Cl as well as the H ions decide the sign of charge of the components of these systems, HNO_3, instead HCl, must be used in adjusting the pH.

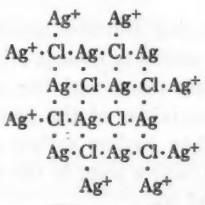
These systems are very complicated, as shown by a single illustration: If a mixture of negative silver chloride and of positive gelatin is negative at pH 4 we know that the silver chloride is in excess and that we can render the system isoelectric by adding more of the gelatin solution. But we can also adopt the activity concept and say that the silver chloride is the most active component for we can also render the system isoelectric either by des-activating the silver chloride with a little $Ag\ NO_3$ or by activating the gelatin by a lowering of the pH with HNO_3. If, on the other hand, the mixture is positive at pH 4 it may be made isoelectric: (*a*) by addng more of the silver chloride, (*b*) by activating the silver chloride by a little NaCl, or (*c*) by des-activating the gelatin by raising the pH.

Now what is the nature of this amphoteric compound (or compounds) of silver and chlorine? To express a series of compounds differing so greatly in their behavior by the simple formula AgCl would be absurd. To say that negative silver chloride represents the compound AgCl, made negative and stabilized by NaCl does not account for its behavior. It would be impossible to account for its reaction with positive gelatin chloride on the assumption that mutual flocculation depends merely on the reaction between the "stabilizing" electrolytes. What reaction can take place between NaCl and HCl?

The following hypothesis will be found to account for all the observed phenomena. When crystals of AgCl are forming in a solution both ions must enter the crystal lattice in the same proportion until one of the ions is depleted, then the crystals must cease to grow. But the other ion, if present in excess, will continue to be attracted by the ions of opposite charge in the surface layer of the crystals. Since the ions which are deposited in excess are only partly neutralized the crystal will carry an electric charge and act as a complex anion or cation which may be represented as follows:



Chlor-argentic anion-complex



Chlor-argentic cation-complex

The ions in excess which retain a free charge must remain paired, the Cl ions to the Na ions, and the Ag ions to the NO_3 ions. But the Na and NO_3 ions possess a high solution tension and remain dissociated forming the outer layer or ion atmosphere. If, in place of the customary molecular formula, we attempt a formulation of a colloidal ionogen of this type we might represent the ionization by the following scheme:

$$(AgCl)_x \cdot (ClNa)_y \rightleftarrows {}_yNa^+ + (AgCl)_x \cdot Cl_y^-$$

and

$$(AgCl)_x \cdot (AgNO_3)_y \rightleftarrows {}_yNO_3^- + (AgCl)_x \cdot Ag_y^+$$

If, in the preparation of negative silver chloride we use HCl instead of NaCl we would obtain the acid form of the complex $H_y(AgCl)_xCl_y$. This compound is analogous to chlor-auric acid. The complex cyanides of silver are other examples of this type of compounds, the main difference being that these complex ions are soluble.

The values of x and y will depend upon the excess of either reagent. With a large excess of NaCl or $AgNO_3$ the negative or positive charge will be strong and the crystals will remain small. This means a large surface and a relatively large value of y.

When negative silver chloride reacts with positive gelatin chloride a double displacement takes place, NaCl is formed and the chlor-argentic anion unites with the gelatin cation. When positive silver chloride reacts with negative gelatin, i.e., Na-gelatinate, $NaNO_3$ is formed and the chlor-argentic cation unites with the gelatin anion.

THE REACTIONS WITH BARIUM SULFATE

The cataphoresis of barium sulfate has been shown in a previous publication (6). With equivalent quantities of $BaCl_2$ and an alkali sulfate a weakly electropositive precipitate is formed. This is taken to indicate that the Ba ions are more strongly associated in the crystal lattice than the SO_4 ions. An excess of Ba results in a still more positive precipitate, whereas an excess of SO_4 ions yields a negative charge. The positive and negative barium sulfate crystals may be respectively represented as follows:

$(BaSO_4)_x \cdot (Ba)_y^{++}$	$(BaSO_4)_x \cdot (SO_4)_y^{--}$
cation complex	anion complex

The accompanying diffusible anions and cations are displaceable and may consist of any anions or cations originally present in the solution.

Barium sulfate reacts with the proteins in the same manner as silver chloride but the activities of the two salt complexes differ. Silver chloride is more active than barium sulfate and each of the two compounds is more active in the positive than in the negative condition. This is clearly shown in tables 85 and 86.

Table 84 shows that cataphoresis of "free" silver chloride. The isoelectric point could not be determined because in equivalent proportions of $AgNO_3$ and

TABLE 84

The $AgNO_3$ + NaCl system (50 cc.)

$AgNO_3$, 0.1 *N*	NaCl, 0.1 *N*	FLOCCULATION		μ/SEC. 1 VOLT/CM.	pH
		After mixing	Overnight		
cc.	*cc.*				
2.0	1.5	Opal	+	+2.9	....
2.0	1.9	Opal	++	+2.5	....
2.0	1.95	Opal	+++	Some + Some −	6.3
2.0	2.0	Milky	++++	Some + Some −	6.35
2.0	2.05	Milky	+++	−2.5	6.4
2.0	2.1	Opal	+	−3.0	....
2.0	2.5	Opal	0	.....	....

TABLE 85

NaOH OR HNO_3	FLOCCULATION		μ/SEC. 1 VOLT/CM.	pH
	After mixing,	Overnight		
millimol				
The $(AgCl)_x \cdot (AgNO_3)_y$ + Na-caseinate system (in 50 cc.)				
A: 2 cc. 0.1 *N* $AgNO_3$ (+ HNO_3).				
B: 1 cc. 0.1 *N* NaCl + 5 mgm. Na-caseinate (+ NaOH).				
0.025 NaOH	Opal	+	−1.32	8.2
0.015 NaOH	Slow	++++	−0.89	7.5
0.01 NaOH	Instant	++++	−0.43	7.2
0.0	Slow	++++	+0.61	6.5
0.005 HNO_3	Milky	+++	+1.01	5.8
0.01 HNO_3	Opal	0	.....	4.5
The $(AgCl)_x \cdot (ClNa)_y$ + Na-caseinate system (in 50 cc.)				
A: 10 cc. 0.1 *N* $AgNO_3$.				
B: 15 cc. 0.1 *N* NaCl + 5 mgm. Na-caseinate (NaOH).				
0.01 HNO_3	Opal	0	.....	6.0
0.02 HNO_3	Slow	++++	−1.38	4.4
0.03 HNO_3	Rapid	++++	+ slight	4.0
0.04 HNO_3	Rapid	++++	+0.38	3.8
0.05 HNO_3	Rapid	++++	+0.61	3.6
0.10 HNO_3	Rapid	++++	+0.95	3.1

NaCl and in a slight excess of the former the particles migrated in opposite directions. It seemed as if the larger aggregates were predominantly positive

while the smallest were negative. It will be noted that the pH is displaced to the acid side by the positive complex and to the alkaline side by the negative complex. The same observation was made in the barium sulfate system (6). This is explained as follows: In the positive systems the diffusible and displaceable anions are partly displaced by the OH ions thus:

$$(AgCl)_x \cdot (AgNO_3)_y + OH^- - H^+ \rightleftarrows$$
$$(AgCl)_x \cdot (AgOH)_y + NO_3^- + H^+$$

TABLE 86

HCl	FLOCCULATION		μ/SEC. 1 VOLT/CM.	pH
	After mixing	Overnight		
millimol				
The $(BaSO_4)_x \cdot (BaCl_2)_y$ + *Na-caseinate system (in 50 cc.)*				
A: 2 cc. 0.1 *N* $BaCl_2$ (+ HCl). B: 1 cc. 0.1 *N* Na_2SO_4 + 5 mgm. Na-caseinate.				
0.005	None	+	−1.68	6.1
0.01	Slow	+++	−0.82	5.4
0.0125	Slow	+++	+0.67	4.5
0.015	Slow	++	+1.60	4.2
0.02	None	+	+2.08	4.1
0.025	None	+	+2.33	4.0
The $(BaSO_4)_x \cdot (SO_4Na_2)_y$ + *Na-caseinate system (in 50 cc.)*				
A: 10 cc. 0.1 *N* $BaCl_2$ (+ HCl). B: 15 cc. 0.1 *N* Na_2SO_4 + 5 mgm. Na-caseinate.				
0.015	None	0	−2.02	6.8
0.02	Slow	++	−1.89	6.0
0.025	Rapid	++++	±0.0	4.5
0.03	Rapid	++++	+0.87	4.1
0.035	Rapid	++++	+0.87	4.0
0.04	Rapid	++++	+1.04	3.8
0.05	Slow	++++	+1.21	3.4

In the negative systems the diffusible and displaceable cations are partly displaced by the H ions in solution thus:

$$(AgCl)_x \cdot (ClNa)_y + H^+ + OH^- \rightleftarrows$$
$$(AgCl)_x \cdot (ClH)_y + Na^+ + OH^-$$

With neutral salts (other than chlorides) the positive complex yields an exchange alkalinity, whereas an exchange acidity is developed by the negative complex. The point of exchange neutrality will, however, vary with the ionic composition of the solution.

As examples of the reactions between silver chloride and barium sulfate on

the one side and the proteins on the other, only the caseinated systems will be shown here because the barium sulfate did not flocculate with gelatin as did silver chloride.

It will be noted that the systems in which the silver chloride and barium sulfate act as cationic complexes, i.e., are positive, are taken 10 times as dilute as the systems in which these compounds act an anionic complexes. Systems containing 15 cc. 0.1 *N* $AgNO_3$ to 10 cc. 0.1 *N* NaCl and 15 cc. 0.1 *N* BaCl to 10 cc. 0.1 *N* Na_2SO_4 together with 5 mgm. casein remained positive at all pH values up to 7.2 and 8.6 respectively. The concentrations of the positive silver chloride and barium sulfate were therefore reduced to one-tenth of the concentrations of the negative suspensions, with the result shown in tables 85 and 86. It must be pointed out that the dilution is not the only difference in the two pairs of systems since the excess of NaCl and Na_2SO_4 amounts to 5 cc. 0.1 *N* while that of $AgNO_3$ and $BaCl_2$ is only 1 cc. 0.1 *N*. The negative silver chloride and barium sulfate complexes were therefore not only more concentrated but were also favored by a higher concentration of the activating ions.

The results were a displacement of the isoelectric pH of 4.8 of "free" casein, to about 4.1 by the negative silver chloride, to 4.5 by the negative barium chloride, to about 6.9 by the positive silver chloride, and to about 4.9 by the positive barium chloride. The zones of flocculation were correspondingly displaced.

This all indicates that silver chloride is more active than barium sulfate. We might account for this behavior by saying that the Ag and Cl ions possess a stronger power of association than do the Ba and SO_4 ions. The "excess association" of Ag or Cl ions on the surface of silver chloride crystals will, at a given excess of either ion in solution, be greater than the "excess association" (i.e. adsorption) of Ba or SO_4 ions on the surface of barium sulfate crystals.

As to the greater activity of the positive than of the negative complexes of the two salts, no definite statement can be made, for we might equally well explain the behavior by saying that, over the pH range in question, the negative protein is less active than the positive protein.

THE CHEMISTRY OF PRECIPITATES

The idea that a precipitate represents a definite compound which is chemically inactive and which can react only through its dissolved phase must be rejected. X-ray analysis has shown that the space arrangement within the crystal lattice is that of an equidistant position of single ions; no one pair of ions can be singled out as a molecular species. This being the case it is only rational that the crystal should behave as an ionic species and react with the ions and polar molecules in the external medium by virtue of the free fields of the surface layer of ions. An ion in the solution which forms a strongly associated compound with one of the ions in the crystal will be attracted and "adsorbed" by the crystal. This creates a potential difference which becomes greater the further the association or adsorption of the ion proceeds. The crystal becomes now an active ion complex with a charge of the same sign as

that of the ion associated in excess. Thus an excess of Ag or Cl ions will become associated with the silver chloride crystals when either ion is present in excess in the solution. Similarly the Ba or SO_4 ions will associate themselves with the barium sulfate crystals.

The potential of the crystals is thus increased until a critical maximum is attained. The critical potential appears to be at about 70 millivolts or about that calculated by von Hevesy (2) for the common single ions. At this potential any oppositely charged ion in the solution will be attracted to the crystal lattice no matter how high the dissociation constant of the compound in the free state. Thus the chlor-argentic cation complex $(AgCl)_x \cdot Ag_y^+$ will attract the NO_3 ions to form the complex $(AgCl)_x \cdot Ag_y^+ \cdot (NO_3)_{y-z}^-$ with z positive charges, where z represents the number of charges at the critical potential. The ion complex is so highly multivalent that the potential would attain enormous magnitudes if all the diffusible ions dissociated. This is apparently why the saloids (compounds between ionic complexes and single diffusible ions) undergo only a limited dissociation (7) as compared to ordinary salts which can undergo complete dissociation since their ions possess only one or two charges and therefore never exceed the critical potential. (The smaller ions are, as we assume, stabilized by the attraction of water molecules, i.e., hydration.)

When the potential of the ionic complexes approaches a maximum a further increase is retarded not only by the attraction of the otherwise easily dissociated diffusible ions but also by an attraction of the polar water molecules. The hydration, i.e., the electrically attracted water, is greater the higher the potential.

The magnitude of the charge and the hydration accounts for the high degree of dispersion of strongly positive or negative precipitates. The oriented layer of water molecules may be assumed to inhibit the growth of the crystals and the charge may conceivably affect the growth as explained in section III (9). Since the free energy of the system must strive toward a minimum it is obvious that the total surface for the distribution of the charge must tend to remain large whenever one ion is associated in excess of an oppositely charged ion. According to the views presented in section III very small particles having a great curvature will accomodate per unit surface, at a given potential, more ions than larger particles. Crystals or colloidal particles which are strongly positive or negative attain accordingly only submicroscopic dimensions.

The highly charged ion complex, whether crystalline or amorphous particles, will actively combine with ions or other ion complexes of opposite charge with which non-dissociated complex is formed. The original charge may then be completely neutralized or there may be a change in charge depending upon the quantity or activity of the added substance. The reaction must be looked upon as a double displacement, but a stoichiometrical relationship must be accidental and in most cases highly improbable. Cation must, however, displace cation, and anion must displace anion in equivalent quantities in order to maintain electroneutrality of the micelle as a unit.

THE FUNCTION OF THE PROTEINS IN THE SOIL

There can be no doubt that the proteins enter into the make-up of the soil colloidal complex, combining with the basic and acidic constituents according to the pH of the soil solution. But the reactions in the soil are so complex that a discussion of this subject must be postponed until a comprehensive study in this direction has been made of the different horizons of the various soil profiles. Such work is now in progress at the New Jersey Agricultural Experiment Station. The preceding study of simple systems must be looked upon as a necessary forerunner to the study of more complicated systems such as those of the various types of the natural soils.

The behavior of the proteins may account for the high nitrogen content in humus and in the B horizons of soils. Proteins, coagulated in the form of various complexes such as those here studied, may be more resistant to decomposition just as leather, which may be looked upon as "protein tannate," is highly resistant. The use of aluminum salts as "tanning" agents indicates a similar resistance of the aluminum "proteinates." In this connection it is of interest to note that the "silicate of gelatin" prepared by Thomas Graham (1) containing 100 parts of silicic acid to about 92 of gelatin "does not putrify in the humid state."

SUMMARY

This paper deals with the electrokinetic and chemical behavior of aluminum- and ferric-"proteinates" and of protein-"humates," "bentonates," and "silicates." The proteins studied include albumin, casein, edestin, and gelatin.

The complex formation between the proteins on the one side and the positive and negative silver chloride and barium sulfate on the other has also been studied and discussed.

The fundamental principles previously established in the case of the silicated, phosphated, and humated compounds of the sesquioxides were found to apply to all the complexes herein described and have thus been generalized.

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NEWS NOTES

The Chilean Nitrate of Soda Nitrogen Research Award of $1,000 was this year divided equally between Dr. W. H. Pierre of the department of agronomy of the University of West Virginia and Dr. Hans Jenny of the department of soils of the University of Missouri. This award has as its object recognition and encouragement of research on any of the diverse aspects of the rôle of nitrogen in economic crop production. It is administered by a standing committee of the American Society of Agronomy.

The award to Dr. Pierre was made in recognition of his work on the effect of the various nitrogenous fertilizers on soil reaction. His work has clearly shown that the continued use of $(NH_4)_2SO_4$ on light, poorly buffered soils will develop within a few years a degree of acidity which is distinctly harmful to many crops. Similar effects are not obtained with the other common nitrogen fertilizers. By supplementing $(NH_4)_2SO_4$ with sufficient lime, satisfactory results may be obtained.

The award to Dr. Jenny was made in recognition of his work on the effect of climate on the nitrogen content of soils. By a careful correlation of climatic data and of the thousands of nitrogen determinations made on the soils of America, Dr. Jenny has been able to develop an equation of state which shows the general relationship existing between the stable nitrogen content of soils and temperature and rainfall, which are the principal climatic factors affecting it.

In addition to the cash awards, the winners were presented with an appropriate certificate by the American Society of Agronomy.

Members of the International Society of Soil Science are urgently requested to send payments for their 1932 dues at once to Dr. A. G. McCall, Room 125 East Wing, U. S. Department of Agriculture, Washington, D. C. Early in 1932 all mailing lists for publications will be closed and only those members whose payments for dues have reached the office of the general secretary abroad by that time, will be enabled to receive without charge, the Journal and Soil Research as well as volume A of the Proceedings of the Sixth Commission meeting at Groningen, 1932. You cannot afford to miss the opportunity to obtain the Transactions of the Second Congress held in Russia, at the special price accorded to members. So please send your remittance promptly.